

# Clinical assessment of arterial stiffness

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A thesis submitted to the University of Birmingham for  
the degree of Doctor of Medicine.

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September 2009

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# Abstract

Increased arterial stiffness is associated with ageing, cardiovascular disease diabetes and renal failure.

The aims of this thesis were to investigate the reproducibility of a tissue Doppler imaging (TDI) based ultrasound system to calculate indices of arterial stiffness, and to investigate changes in arterial wall stiffness in subjects with increased age, peripheral arterial disease (PAD), chronic kidney disease (CKD) and angiotensin converting enzyme inhibitors (ACE-Is).

A reproducibility study demonstrated good reproducibility. A study of healthy subjects demonstrated a stronger relationship with age for arterial stiffness than intima media thickness (IMT). Case control studies investigating changes in subjects with PAD and CKD demonstrated a greater increase in arterial stiffness than IMT when compared to healthy controls. An epidemiological study, investigating the effect of anti-hypertensives on collagen turnover, suggested an association between increased collagen turnover and ACE-Is. Investigating the effect of ACE-I administration on arterial stiffness in subjects with PAD, we demonstrated an increase in collagen turnover and a decrease in arterial stiffness.

We have demonstrated that the TDI-based system is a reproducible method of measuring arterial stiffness. We suggest that arterial stiffness increases more than IMT with ageing, PAD and CKD and that it may increase before cardiovascular disease develops.

## Abbreviations and Acronyms

<b>A2RB</b>	angiotensin II receptor blocker
<b>ABPI</b>	ankle brachial pressure index
<b>ACE</b>	angiotensin converting enzyme
<b>ACE-I</b>	angiotensin converting enzyme inhibitor
<b>ADC</b>	arterial diameter change
<b>ADW</b>	arterial distension waveform
<b>AGE</b>	advanced glycation end product
<b>APW</b>	arterial pressure waveform
<b>AT1</b>	angiotensin type 1
<b>AWM</b>	arterial wall motion
<b>BCCOV</b>	between cycle coefficient of variability
<b>CCA</b>	common carotid artery
<b>CAD</b>	coronary artery disease
<b>CKD</b>	chronic kidney disease
<b>COPD</b>	chronic obstructive pulmonary disease
<b>CVA</b>	cerebrovascular accident
<b>CVD</b>	cerebrovascular disease
<b>DBP</b>	diastolic blood pressure
<b><i>E</i></b>	Young's modulus
<b>ECM</b>	extracellular matrix
<b>eNOS</b>	endothelial nitric oxide synthase
<b><i>Ep</i></b>	pressure strain elastic modulus
<b>ESRD</b>	end stage renal disease
<b>GFR</b>	glomerular filtration rate
<b>HDL</b>	high density lipoprotein
<b>HMG-CoA</b>	3-hydroxy-3-methylglutaryl-coenzyme A
<b>I-CAM</b>	intracellular adhesion molecule
<b>IMT</b>	intima media thickness
<b>LDL</b>	low density lipoprotein
<b>MADC</b>	mean arterial diameter change
<b>MAP</b>	mean arterial pressure
<b>MMP</b>	matrix metalloproteases
<b>MRI</b>	magnetic resonance imaging
<b>NO</b>	nitric oxide
<b>NOS</b>	nitric oxide synthase
<b>ox-LDL</b>	oxidised low density lipoprotein
<b>PAD</b>	peripheral arterial disease
<b>PAI-1</b>	plasminogen activator inhibitor type 1
<b>PP</b>	pulse pressure
<b>pre-pro-ET-1</b>	pre-pro-endothelin-1
<b>PWV</b>	pulse wave velocity
<b>RAAS</b>	renin angiotensin aldosterone system
<b>RAS</b>	renal artery stenosis
<b>ROI</b>	region of interest
<b>SAC</b>	systemic arterial compliance
<b>SBP</b>	systolic blood pressure
<b>TDI</b>	Tissue Doppler imaging
<b>t-PA</b>	tissue plasminogen activator
<b>VSMC</b>	vascular smooth muscle cells

## Dedication

I would like to dedicate this thesis to my wife Anna, and our daughter Lauren. Thank you for putting up with the distant tapping of a keyboard in the spare room when I should have been busy with you instead.

# Acknowledgements

The staff at Heartlands Hospital, Birmingham made this thesis possible.

In particular I would like to thank the members of the Department of vascular surgery for advice and support, notably Gareth Bate for teaching me to scan and collecting data, Emma Burke and Ellen Drew for subject recruitment, and Teun Wilmink and Andrew Bradbury, my supervisors, for advice and support from start to finish.

I would also like to thank Jenny Claridge for proof reading and editorial advice on layout and style.

Dr Pete Hoskins in the department of Medical Physics at Edinburgh University provided invaluable expert advice freely and promptly.

I would also like to thank Terry Hayes from Philips ATL for help with setting up the HDI500 Ultrasound machine.

The Huntingdon Aneurysm Screening project was supported by a grant from the British Heart Foundation.

## Publications

*Arterial stiffness has a stronger relationship with age than IMT or pulse pressure in healthy subjects.* M.W. Claridge, G.R. Bate, P.R. Hoskins, D.J. Adam, A.W. Bradbury, A.B. Wilmkink. **Submitted Atherosclerosis 2009**

*Arterial stiffness in with renal disease: are changes in the vessel wall earlier markers of cardiovascular disease than IMT and pulse pressure?* M.W. Claridge, G.R. Bate, P.R. Hoskins, D.J. Adam, A.W. Bradbury, A.B. Wilmkink. **Submitted Kidney International 2009**

*Measurement of arterial wall stiffness in subjects with vascular disease: are vessel wall changes more sensitive than increase in Intima Media thickness?* M.W. Claridge, G.R. Bate, P.R. Hoskins, D.J. Adam, A.W. Bradbury, A.B. Wilmkink. **Atherosclerosis 2009:205(2):477-480**

*A reproducibility Study of a TDI based method to calculate indices of arterial stiffness.* MW Claridge, GR Bate, JA Dineley, PR Hoskins, T Marshall, DJ Adam, AW Bradbury and AB Wilmkink. **Ultrasound in Medicine and Biology 2008:34(2):215-20.**

*ACE inhibitors increase type III collagen synthesis: a potential explanation for reduction in acute vascular events by ACE inhibitors.* Claridge M.W., Hobbs S. D., Quick C., Day N., Bradbury A.W., Wilmink A.B.M. ***European Journal of Vascular and Endovascular Surgery* 2004; 28:67-70**

*Tissue Doppler imaging is a novel and reproducible method for defining the arterial distension waveform and calculating indices for arterial elasticity.* Claridge M, Bate G, Adam D, Hoskins P, Bradbury A, Wilmink T. ***Journal of Ultrasound in Medicine*, 2006; 25: S36**

*Increased Arterial Stiffness in Peripheral Vascular Disease: Arterial walls get Thicker but do not change in Structure.* Claridge M, Bate G, Adam D, Hoskins P, Bradbury A, Wilmink T. ***Journal of Ultrasound in Medicine*, 2006; 25: S19**



## Presentations

*Tissue Doppler imaging is a novel and reproducible method for defining the arterial distension waveform and calculating indices for arterial elasticity.* Claridge M, Bate G, Adam D, Hoskins P, Bradbury A, Wilmink T. **Association of Ultrasound in Medicine, Washington 2006**

*Increased Arterial Stiffness in Peripheral Vascular Disease: Arterial walls get Thicker but do not change in Structure.* Claridge M, Bate G, Adam D, Hoskins P, Bradbury A, Wilmink T. **New Investigator Prize Session Association of Ultrasound in Medicine, Washington 2006**

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## BASIC PRINCIPLES OF ARTERIAL STIFFNESS

*Arterial stiffness is a generic term that describes the rigidity of the arterial wall. The arterial wall is structurally made up of three layers, with varying degrees of elasticity which is present to counter the pulsatile ejection of blood from the heart. The majority of research in arterial stiffness is based on elastic theory. The application of elastic theory to arterial stiffness assumes that the arterial wall is heterogeneous and moves isotropically. Whilst this may not be the case, these assumptions have been allowed as research into arterial stiffness would otherwise not be practicable on a clinical level.*



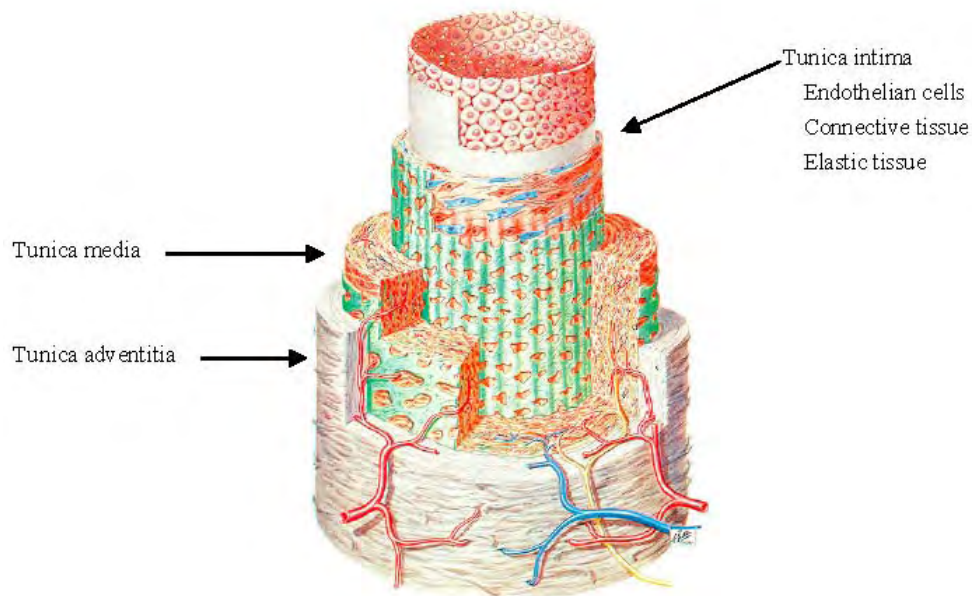
## **Definition of arterial stiffness**

Large arteries are not just simple conduits for blood. In addition to this function, they also act as a buffer to the pulsatile blood flow from the heart. This role is fulfilled via the elastic nature of the arterial walls. Detailed assessment of the arterial tree requires information about the form of the arterial pulse (i.e. the arterial pressure waveform) and information about how the arterial walls move in reaction to this waveform (i.e. the arterial distension waveform). At the most basic level, palpation of an arterial pulse can convey a crude impression of these two parameters. Records of the clinical importance attached to the qualitative assessment of the pulse have been present for nearly two hundred years.<sup>1</sup> Qualitative assessment declined in the 19<sup>th</sup> century with the development of accurate sphygmomanometry, collecting quantitative information about the extremes of the pulse.<sup>2,3</sup> In the past three decades as new technology and understanding has become available, attention has once more focused upon the qualitative assessment of the arterial waveforms and their potential clinical relevance. Many terms exist in this field, often with slightly different interpretations and commonly referring to a distinct physical equation or property. Arterial stiffness has been chosen as a generic term in this thesis to avoid confusion and describes the describes the rigidity of arterial walls.<sup>4</sup>

## Arterial structure

All arteries are composed of three layers, the intima, media and adventitia. The intima is the innermost layer, a single layer of endothelial cells and associated connective tissue. The middle layer is the media which is composed of a varying amount of elastic and smooth muscle tissue depending on the size and location of the artery in the arterial tree. The outer layer is the adventitia which is fibrous connective tissue.

**Figure 1:** Structure of a medium sized artery.



(after Kangasniemi & Opas)<sup>5</sup>

## Mechanical principles

A force (stress) acting on a solid body at rest causes parts of the body to move relative to each other (strain). At the end of the application of the

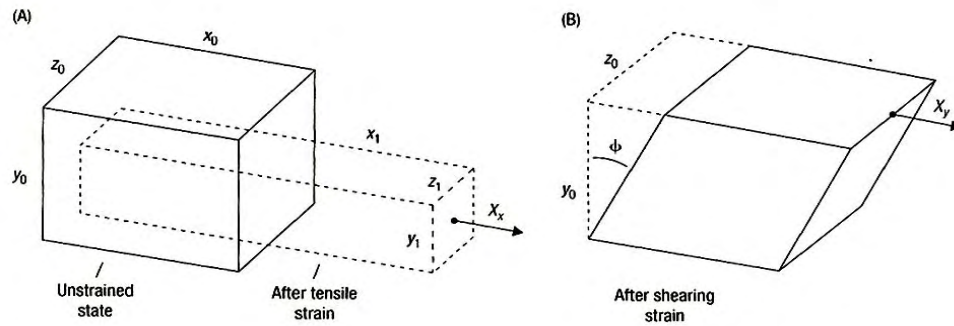
force, if the body regains its original form, then it is said to be elastic. If the body retains the deformation caused by the force acting upon it then it is said to be plastic.

### ***Strain***

Strain is described as the ratio of the deformation of a body compared to its original form and thus has no units. Longitudinal strain is defined as the change in length of a body in response to stress. An increase in length is positive strain and a decrease in length is negative strain. Compressive strain refers to a change in volume of a body and shear strain to angular deformation, a displacement of two points in parallel planes in a direction parallel to those planes.<sup>6</sup> As demonstrated in Figure 2, a body that is extended longitudinally (longitudinal strain) will at the same time get shorter transversely (transverse strain).

The ratio of transverse to longitudinal strain is called the Poisson ratio ( $\sigma$ ).  $\sigma$  for a particular material is constant under small strain. The effective range for  $\sigma$  is 0 to 0.5. In a small extension of a material with a ratio of 0.5, the volume of the material remains the same when stretched. This is known as iso-volumetric deformation, and it is exhibited by rubber-like substances where  $\sigma = 0.48$ .<sup>7</sup>  $\sigma$  for the arterial wall is believed to be close to 0.5.<sup>8</sup>

**Figure 2:** Longitudinal (or tensile) strains.



**Legend:** (A) An elastic solid of dimensions  $X_0$ ,  $Y_0$ ,  $Z_0$  in the unstrained state before deformation (solid lines) and after a positive tensile strain  $\epsilon_{xx}$  resulting from the tensile stress  $X_x$  (broken lines). Strains in the  $y$ -axis and  $z$ -axis are  $\epsilon_{yy}$  and  $\epsilon_{zz}$  respectively and are both negative. (B) Shearing strain produced by the stress  $X_y$ . One measure of strain is the angle of shear,  $\Phi$ . (After McDonald)<sup>6</sup>

However it cannot be assumed that the Poisson ratio in all planes of a material is similar. Considering a cube shaped section within the body of a material it can be shown that there are six independent components of strain.<sup>9</sup> If the material has the same elastic properties in all planes then it is said to be isotropic. Conversely if the elastic properties are not the same the material is said to be anisotropic. If the elastic properties in each direction are not the same but however the material is iso-volumetric under strain then the average values of  $\sigma$  must be equal to 0.5.

### **Elastic theory**

Elastic theory attempts to explain the relationship between the force applied to a body and its consequent change of deformation. The behaviour of a body in response to a stress distinguishes solid from liquid

as a liquid will undergo viscous flow whereas a solid will not. However a large number of bodies are termed viscoelastic as they exhibit qualities attributable to both an elastic solid and a viscous liquid; their behaviour in any given situation depends on the size of the stressing force and the rate at which it is applied. Arterial walls are viscoelastic.<sup>6</sup>

Hooke's law<sup>10</sup> dictates that the deformation is proportional to the force applied and this holds true in an elastic body until it reaches its elastic limit which is the point at which the force applied to a body is so great that it cannot regain its original form. Further increase of load would result in the yield point being reached where the body will break without a further increase in force. The application of elastic theory on a solid body can only be valid below the elastic limit of that body, and also requires that the material structure of that body is homogeneous and that any deformations as result of the applied force are minute (that the body returns to its original form when the force is removed). These two assumptions are clearly not valid when considering arterial walls. Arterial walls retain large deformations, and as discussed previously are composed principally of collagen, elastin and smooth muscle which all have different elastic properties. Despite these limitations, most work on arterial mechanics uses elastic theory as its basis, as this is the simplest model that is most easily applied to arterial wall movement.

## ***Stress***

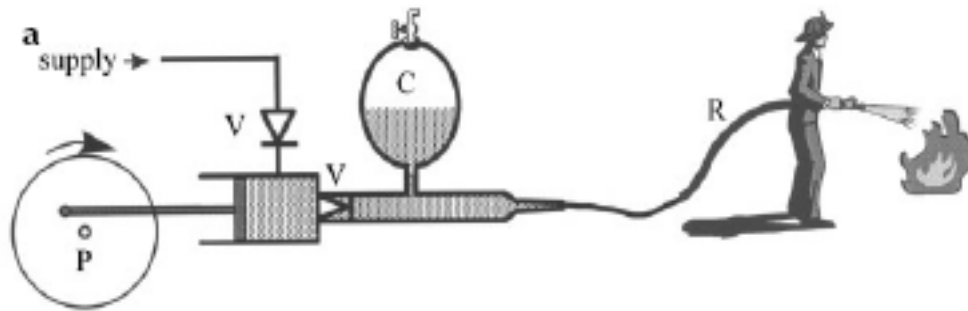
Stress is the intensity of a force acting over a given area of a body and thus the units of stress are force per unit area. The effect of stress on a point in a plane can be described by forces acting in parallel to the axis and acting tangentially to the axis. These forces can be resolved into six independent components of stress. Given that Hooke's law<sup>10</sup> demonstrates that strain is proportional to stress, in an anisotropic material there are 36 constants of proportionality as there are six components of strain and six components of stress acting at any one point on a body. Fifteen of these constants can be shown to be interrelated. Thus in an anisotropic material, 21 constants need to be considered. An isotropic material however has the same elastic behaviour in all three axes and thus the number of constants of proportionality becomes 2 instead of 21. It is immediately apparent therefore why arterial walls are assumed to be isotropic even though structural analysis demonstrates different macro- and microscopic components.

## ***Circulatory model***

One of the earliest and simplest models for the arterial system was first published by the Rev Stephen Hales in his book entitled *Haemostaticks* in 1733.<sup>11</sup> This model demonstrates how the major arteries act as a cushion or buffer to the intermittent pumping of the heart. Combining this property with the high peripheral resistance of the arterioles, this explains how the flow of blood at the tissues is near constant and virtually devoid of cardiac

pulsation. This model (shown in Figure 3) is termed the Windkessel model after the mediaeval German fire compression chamber which illustrated the German translation of his work.

**Figure 3:** Diagram of the Windkessel model for the arterial circulation.



**Legend:** A pulsatile pump, pumping into a pressure vessel partly filled with air which thus acts as a capacitor. The output is via a high resistance pipe and the result is a near constant outflow despite the pulsatile inflow. P= pulsatile pump; V=one way valve; C=capacitor; R= high resistance pipe. (after Greenwald)<sup>12</sup>

Clearly the properties and function of the arterial system are much more complex than this. In particular, the approximation together of total arterial compliance and total peripheral resistance is a gross simplification which does not reflect anatomical knowledge of the arterial tree. Nevertheless the Windkessel model does help to explain how arterial elasticity fits into the function of the arterial system under normal physiological conditions.

The circulation actually distributes cardiac output via a series of branching networks, and this model serves to explain the concept of wave reflection. At every branching of an artery, a small proportion of the forward travelling pulse wave is reflected backwards. Thus the arterial waveform is a summation of both the forward travelling pulse wave and

the reflected wave travelling back towards the heart. This quality may have a significant effect on systolic blood pressure,<sup>13</sup> and perhaps explain the systemic cardiovascular changes that are found in subjects with peripheral arterial disease (PAD).

## **Conclusions**

Arterial stiffness is a generic term that describes the rigidity of the arterial wall. The physiological reason for the elastic nature of the arterial wall is to buffer the pulsatile ejection of blood from the heart and to provide near constant flow in the capillary beds. The mechanical principles behind arterial stiffness are complex but are based on the relationship between stress and strain. In the case of arterial stiffness, the stressing force is the pressure of pulsatile blood flow and the resulting strain is the change in length of the arterial wall. Most work on arterial stiffness is based on elastic theory despite the fact that this requires the body studied to be elastic rather than viscoelastic as is the case with the arterial wall. In addition, the arterial wall is considered to be isotropic in response to stress as this permits much simpler calculation of indices of arterial stiffness.



## INDICES OF ARTERIAL STIFFNESS

*All indices to calculate arterial stiffness require information about simultaneous change in pressure and distension of the target artery. There are many defined indices, with relative advantages and disadvantages. Most modalities used to collect data are non-invasive. The most widely accepted method is aortic pulse wave velocity collected using applanation tonometry. Indices of arterial wall stiffness can be collected using ultrasound which may have the greatest application in research settings comparing them to IMT.*

## Introduction

There are many indices of arterial stiffness. All require information about simultaneous change in arterial size and arterial pressure in order to quantify the change in arterial stiffness. The change in arterial size may be calculated as the change in diameter or change in volume. Both of these parameters should be measured at the same site in the arterial tree due to discrepancy in values across the arterial tree, although *in vivo* this may be practically impossible.<sup>14</sup> As in most cases of equipoise, the existence of multiple indices for quantifying arterial stiffness reflects the fact that none of them are superior to one another; all have inherent advantages and disadvantages.

## Pulse pressure

Pulse pressure (PP) is the difference between systolic and diastolic blood pressure. It has long been recognized as a valuable surrogate marker of arterial stiffness<sup>15</sup> as it depends on cardiac output, large artery stiffness and wave reflection. Both systolic and diastolic blood pressure increase with age. However, beyond the sixth decade, diastolic blood pressure does not increase whereas systolic blood pressure continues to do so and thus pulse pressure increases with age.<sup>13</sup> Pulse pressure takes no account of change in volume and therefore is not a true measure of arterial stiffness. Moreover, most measures of pulse pressure are made from the brachial artery using an oscillometric sphygmomanometer.

These measurements may not accurately reflect central pulse pressure, with differences of up to 20 mmHg noted.<sup>16,17</sup> Nevertheless, data from the Framingham study demonstrates that pulse pressure predicts the risk of coronary heart disease better in a population over the age of 50 years than systolic or diastolic blood pressure measurements alone.<sup>18</sup>

## **Arterial compliance and distensibility**

Arterial compliance is defined as a change in volume for a given change in pressure and is defined by the following formula:

$$\Delta d / \Delta p \text{ m/KPa}$$

Arterial distensibility is defined as compliance divided by the initial volume:

$$\Delta v / (\Delta p \times v) \text{ KPa}^{-1}$$

Where  $v$  = volume

When considering pulsatile flow (which is obviously the case with arteries *in vivo*) many researchers have considered that compliance and distensibility can be estimated using the change in radius, diameter, flow or cross-sectional area for a given change in pulse pressure as long as both of these variables are measured at the same site in the arterial tree.<sup>19,20</sup> Various other estimations of compliance and distensibility using

stroke volume and pulse pressure have been demonstrated to be inaccurate and have been discredited.<sup>21</sup>

## **Elastic moduli**

The relationship between stress and strain is expressed as an elastic modulus. All elastic moduli express the units of stress, as strain is a ratio and therefore has no units.

### ***Pressure strain elastic modulus***

The pressure strain elastic modulus ( $E_p$ ) was first presented by Peterson et al in 1960<sup>22</sup> as a modulus which did not require knowledge of wall thickness in order to be calculated, and is calculated by the following formula:

$$E_p = (\Delta p * d) / \Delta d \text{ KPa}$$

where  $d$  is diameter,  $\Delta p$  is the difference pressure at systole and diastole and  $\Delta d$  is the difference in diameter at systole and diastole.

### ***Young's modulus***

Young's modulus ( $E$ ) is the elastic modulus in the longitudinal direction of a material and was named after Thomas Young.<sup>23</sup> It requires knowledge of wall thickness to be calculated and is defined by the formula:

$$E = (d/2h) * E_p \text{ KPa}$$

Where  $h$  is wall thickness.

There is discrepancy in the literature as to the exact form that  $E$  should take when applied to arterial stiffness. Different editions of the same book even present a different formula. However the author is confident that the formula quoted in this thesis is correct. This statement is reinforced by a literature search and by kind help from the department of Medical Physics at Edinburgh University, whose members have derived the formula from first principles (see Appendix 1).

$Ep$  is regarded as a measure of structural stiffness, describing the elastic behaviour of the artery as a whole and  $E$  as a measure of material stiffness which describes the behaviour of the arterial wall itself.<sup>24</sup>

## Stiffness index

The stiffness index was introduced by Kawasaki et al<sup>25</sup> as an index of arterial stiffness independent of pressure. It is described by the following formula:

$$\ln(p_s/p_d)/(d_s-d_d/d_d)$$

where  $p_s$ = systolic pressure,  $p_d$  = diastolic pressure,  $d_s$ = systolic diameter,  $d_d$ = diastolic diameter

It is based on work by Hayashi et al<sup>24</sup> who found a linear relationship between the logarithm of relative pressure and the distension ratio when

examining isolated human arteries in vitro. Hayashi and colleagues argue that the slope of this exponential function describes the behaviour of the arterial wall within the *in vivo* pressure range without dependence on pressure.

## Augmentation index

The shape or contour of the ascending aortic pressure wave has been classified and has been shown to change in morphology with increasing age.<sup>26</sup> The main change is the point of peak systolic blood pressure and its relationship to the inflection point on the waveform representing the reflected pressure wave. The augmentation index is defined as the ratio of augmented ascending aortic pressure and pulse pressure and can be calculated from the following formula:

$$(p_s - p_i)/(p_s - p_d), p_d)$$

where  $p_i$  is pressure at the wave reflection.

This method of estimating arterial elasticity has been used in several studies.<sup>27</sup> However it has many limitations including erroneous results when an inflection point is not easily identified.<sup>28</sup> In addition the augmentation index is also influenced by heart rate.

## Pulse wave velocity

Pulse wave velocity (*PWV*) is an indirect measurement of arterial stiffness over an arterial segment. It is measured using applanation tonometers such as the Complior (Colson, Paris, France) and the SphygmoCor (PWV Medical PTY Limited, Sydney, Australia). *PWV* is calculated by the following formula:

$$PWV = distance / \Delta t \text{ ms}^{-1}$$

where  $t$  = time

The transit time is measured by two applanation tonometers placed over peripheral pulses and the distance between them is estimated by direct superficial measurement. This estimation is subject to inaccuracy unless the artery between the two pulses is in a straight line and can be difficult to make particularly in the case of obese patients.<sup>21</sup> *PWV* is related to Young's modulus:

$$PWV = \sqrt{(E \times h / 2rp)}$$

where  $p$  is the density of fluid (approx 1.05 for blood).<sup>29</sup>

## Conclusions

There are numerous formulae to calculate arterial stiffness. All require information about change in pressure and size, and are summarised in Table 1.

**Table 1:** Indices of arterial stiffness

Term	Definition	Mode of Measurement
<b>pulse pressure</b>	the difference between systolic and diastolic blood pressure  $p_s - p_d$	sphygmomanometry
<b>arterial compliance</b>	absolute diameter(or area) change for a given pressure step  $\Delta d / \Delta p$ m/KPa	ultrasound MRI
<b>arterial distensibility</b>	relative change in diameter (or area) for a given pressure change, the inverse of elastic modulus  $\Delta d / (\Delta p \times d)$ KPa <sup>-1</sup>	Ultrasound MRI
<b>elastic modulus</b>	the pressure change required for theoretical 100% stretch from resting diameter  $(\Delta p \times d) / \Delta d$ KPa	Ultrasound MRI
<b>Young's modulus</b>	Elastic modulus per unit area  $(\Delta p \times d) / (\Delta d \times h)$ KPa	Ultrasound MRI
<b>augmentation index</b>	difference between the second and first systolic peaks as a percentage of pulse pressure  $(p_s - p_i) / (p_s - p_d)$	pressure waveform
<b>stiffness index</b>	ratio of the log of systolic/diastolic pressures to relative change in diameter  $\ln(p_s / p_d) / (d_s - d_d / d_d)$	ultrasound
<b>pulse wave velocity</b>	velocity of travel of the pulse along a length of artery  $distance / \Delta t$ ms <sup>-1</sup>	pressure waveform ultrasound MRI

**Legend:**  $p$ =pressure;  $d$ =distance;  $h$ =thickness diameter;  $t$ =time;  $_s$ =parameter measured at systole;  $_d$ =parameter measured at diastole;  $p_i$ =pressure wave reflection.



# 3

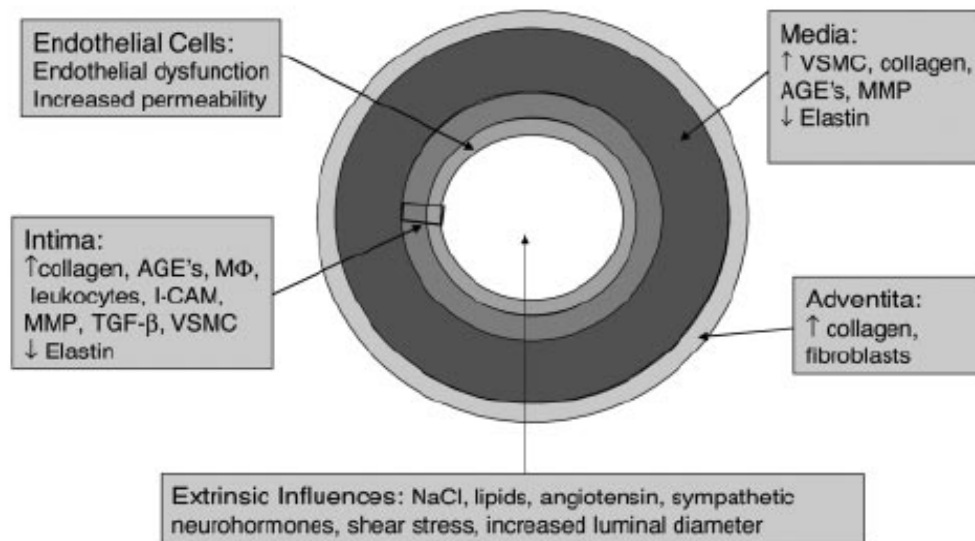
## MECHANISMS OF ARTERIAL STIFFNESS

*Change in arterial stiffness can be mediated by change in either the structural and cellular components of the arterial wall or both. The changes in the structural component of the arterial wall tend to be long term and irreversible, as a result of a change in the quantity and quality of collagen and elastin. The cellular component of arterial stiffness changes as a result of vascular smooth muscle tone and endothelial cell function. This may be part of physiological control of the cardiovascular system and may be reversible. There are many putative genetic loci for genetic causes of arterial stiffness.*

## Introduction

The mechanisms of arterial stiffness are complex and interactive. Stiffness develops due to a combination of reversible and irreversible changes in the structural and cellular elements of the vessel wall, and are summarised in Figure 4.

**Figure 4:** Summary of the multiple causes and locations of arterial stiffness



**Legend:** ↑=increased; ↓=decreased; AGE=advanced glycation end product; MΦ=macrophage; I-CAM=intracellular adhesion molecule; MMP=matrix metalloproteinase; TGF-β= tissue growth factor beta; VSMC=vascular smooth muscle cells. (After Zieman)<sup>30</sup>

These changes are influenced by various haemodynamic forces<sup>31,32</sup> resulting from the flow of blood through the arteries, which results in a patchy periphery sparing distribution of arterial stiffness within the vascular tree,<sup>33-37</sup> and by so-called 'extrinsic factors', notably hormones, salt and glucose regulation. In turn common chronic disease

states such as hypertension, renal failure and diabetes mellitus can exacerbate these mechanisms.

## **Structural changes in arterial stiffening**

The structure and compliance of the arterial wall is dependent on the relative proportions of collagen and elastin, the two major proteins making up the extracellular matrix (ECM). The relative proportion of these two proteins is maintained at near constant levels in health via homoeostasis. Dysregulation, usually stimulated by an inflammatory milieu, leads to decreased production of elastin and overproduction of abnormal collagen, thus resulting in increased arterial stiffness.<sup>38</sup> The main method by which collagen and elastin are degraded is by matrix metalloprotease (MMP) production. MMPs cause degradation of collagen and elastin via their collagenolytic and elastinolytic effects. Thus MMPs degrade the ECM by creating uncoiled collagen and broken and frayed elastin. MMPs are produced by vascular cells and macrophages and polymorphonuclear neutrophils.<sup>39</sup> The potent catabolic effect of MMP is countered by tissue inhibitors of MMPs.<sup>33,40</sup> This role is vital in maintaining collagen and elastin homoeostasis.

The main role of collagen is to provide tensile strength to the arterial wall. Collagen molecules are enzymatically cross-linked soon after production. Damage to these cross-links will cause unravelling of the collagen matrix. The turnover of collagen is slow and susceptible to non-enzymatic ligation cross-linking, resulting in an increased quantity of

collagen with a dysfunctional matrix. Hypertension, manifesting as increased luminal pressure also stimulates excessive collagen production.<sup>41</sup> Elastin is also cross-linked to promote stability. Damage to these cross-links causes weakening of the elastin array and also predisposes to demineralisation by calcium and phosphorus. Together with decreased production and repair of elastin, these factors all result in increased arterial stiffness.<sup>42-44</sup>

Advanced glycation end products (AGEs) arise as a result of non-enzymatic protein glycation. These products form irreversible cross-links in proteins such as collagen with slow turnover.<sup>45,46</sup> AGE-linked collagen is stiffer per se and also less easily degraded, resulting in an increased proportion of this type of collagen in the arterial wall.<sup>47</sup> Elastin molecules are also susceptible to AGE cross-linking, resulting in a less effective elastic matrix.<sup>48</sup> AGE also has an effect on endothelial cell function and inflammatory response, resulting in increased generation of oxidant species, oxidant radical formation, pro-inflammatory cytokines and vascular adhesion molecules.<sup>49,50</sup> These substances can mediate increased vascular stiffness via MMPs, provoke endothelial dysfunction and thus elevated smooth muscle tone, depress endothelial flow mediated dilatation, diminish response to vascular injury, affect angiogenesis and promote atherosclerotic plaque formation.<sup>51-53</sup> These molecular changes are visible on a macroscopic level as increased intima media thickness<sup>54</sup> (IMT) and hypertrophy of the smooth-muscle layer.<sup>55</sup>

On a microscopic level the intima of diseased vessels demonstrate abnormal and disarrayed endothelial cells, an increase in collagen, frayed and broken elastin molecules, infiltration of vascular smooth muscle cells and an increase in macrophages, mononuclear cells, MMPs, cytokines and cell adhesion molecules.<sup>56</sup>

## **Cellular component of arterial stiffening**

As well as changes to the structure of the arterial wall, arterial stiffness can also lead to altered vascular smooth muscle cell (VSMC) tone and endothelial cell function. VSMC tone can be influenced in a number of ways, including mechanical stimulation causing cell stretching and changes in calcium signalling<sup>30</sup> and also by angiotensin II,<sup>57</sup> endothelin,<sup>58</sup> oxidant stress<sup>59</sup> and nitric oxide. The main effect of endothelial dysfunction appears to be an imbalance in production and breakdown of vasodilating and vasoconstricting substances notably nitric oxide, oxygenators and constricting hormones.<sup>60</sup> In particular, levels of nitric oxide may be altered as a result of reduction of expression, increased expression of a natural nitric oxide synthase (NOS) inhibitor, asymmetrical dimethylarginine,<sup>61</sup> and activation of reactive oxygen species by stress, hormones and AGEs.<sup>62</sup>

There is evidence that structural stiffening itself may result in endothelial dysfunction as endothelial cells cultured in expansile tubing and subjected to pulsatile perfusion demonstrate stimulation of

endothelial NOS expression; this expression is not found when the cells are cultured in stiff tubing but still subjected to the same pulsatile flow.<sup>63</sup>

## Genetic implications

Given its multifactorial nature, it will come as no surprise that more than one genetic locus has been identified as associated with arterial stiffening. As has been discussed (summarised in Figure 4), many different proteins have been implicated in the mechanisms of increased arterial stiffness and increased arterial stiffness has been shown in subjects with gene polymorphisms for angiotensin converting enzyme<sup>64</sup> (ACE), angiotensin II type 1 receptor,<sup>65</sup> endothelin A and B receptor,<sup>66</sup> collagen I  $\alpha 1$ ,<sup>67</sup> fibrillin-1,<sup>68</sup> IGF-1,<sup>69</sup>  $\alpha$ -adducin,<sup>70</sup> aldosterone synthase,<sup>71</sup> and MMPs 3 and 9.<sup>72,73</sup>

Mitchell et al. suggest that increased pulse pressure has a moderate heritability in the Framingham heart study population. They performed a genome wide scan which found some correlation with reflective wave amplitude, forward wave amplitude and mean arterial pressure. Variance analysis suggested linkage to genetic loci on chromosomes 1, 2, 4, 7, 8, 13 and 15. This study found no evidence of linkage with previously implicated genetic polymorphisms, which had been identified in small diseased study populations, rather than in a community-based unselected sample.<sup>74</sup>

## Conclusions

Changes in both the structural and cellular components of the arterial wall result in increased arterial stiffness. These changes may be reversible or irreversible. On a structural level, the majority of these changes are irreversible and come about as a result of the change in elastin and collagen homeostasis, largely mediated by MMPs. Changes in the cellular control of arterial stiffness is via altered VSMC tone and endothelial cell function. Limited evidence has been found for genetic causes of arterial stiffness, but given the multifactorial nature of the causes of arterial stiffness and thus the multiple different proteins involved there are many putative genetic loci.

## 4

# CLINICAL IMPLICATIONS OF ARTERIAL STIFFNESS

*Arterial stiffening was just thought to be part of the ageing process, with little clinical significance. However it is now clear that whilst arterial stiffness does increase with age, accelerated arterial stiffening has a strong relationship with many disease states.*



## **Relationship with age**

Qualitative changes in cardiovascular physiology with age have been noted for well over 100 years.<sup>15,75</sup> However interest in the quantitative limits of blood pressure gained favour over the qualitative form with the emergence of the cuff sphygmomanometer. Whilst there is now a much newer body of research with relevance to vascular ageing, it must be regarded with caution. The reasons for this include a lack of an accurate animal model to simulate human cardiovascular ageing,<sup>76-79</sup> inaccuracy of non-invasive investigations, difficulty in discriminating the difference between natural ageing and occult disease, controversy over what the natural ageing process actually is, difficulty in identification of the reserve physiological capability of cardiovascular and other organs, and finally errors resulting from cross-sectional studies that may in fact contain only super fit survivors rather than subjects more susceptible to the effects of ageing who have already fallen by the wayside.<sup>80</sup>

### ***Structural and functional arterial changes with increasing age***

Many physicians regard the increasing prevalence of atherosclerosis with age as a normal part of arterial ageing. However the process they refer to is stenotic and localised and should be regarded as an entity separate from arterial ageing, a process which is diffuse across the arterial tree and characterised by dilation and increased stiffness.<sup>81</sup> Atherosclerosis is predominantly a disease of the intima, and has well recognised localised

sites of affliction within the arterial tree, where as ageing change has a more diffuse pattern and arises in the media. The main effect brought about by atherosclerosis is distal ischaemia as a result of the obstructing atherosclerotic plaque, with the only dilatory effect being found in the distal abdominal aorta. Ageing change causes diffuse arterial wall weakness and thus may present with aneurysmal change anywhere in the arterial tree. Histological studies in different human ethnic groups both prone and not prone to atherosclerosis or hypertension have demonstrated that the age-related changes to the human arterial wall do involve intimal hyperplasia. However the most significant change is in the arterial media. Here there is a loss of the orderly arrangement of elastin fibres which also undergo thinning splitting, fraying and fragmentation. There is also an increase in collagen and ground substance.<sup>82</sup> These changes are not confined to old age, but are progressive throughout life, as are the accompanying functional changes.<sup>76</sup> On a microscopic level, increased amounts of collagen and glycosaminoglycans are found in the aortic wall with ageing, together with a decreasing amount of smooth muscle and structurally normal elastin.<sup>82</sup>

The main functional change with age appears to be progressive stiffening of the central aorta with subsequent effect on cardiac function.<sup>15,83</sup> Increased aortic stiffness with age has been found in both western populations with a high propensity towards atherosclerosis and other populations not prone to atherosclerosis.<sup>84-88</sup> It is therefore believed to be a genuine age dependent change. At any particular age there is a

wide range of arterial stiffness.<sup>89</sup> This finding appears only to hold for the aorta and other central elastic arteries, with little evidence being found for a change in stiffness with age in the muscular arteries such as the brachial, radial or femoral.<sup>36, 86, 90</sup>

## **Relationship with lifestyle and disease**

### ***Arterial stiffness and atherosclerosis***

The association between increased arterial stiffness and atherosclerosis is clear<sup>91,92</sup> and has been shown in humans,<sup>93-95</sup> nonhuman primates<sup>96</sup> and other mammals.<sup>97,98</sup> Looking at apolipoprotein E knockout (apoE-KO) mice, Wang et al have shown that these mice develop moderate atherosclerotic lesions in the aortic arch at four months old. These lesions increased significantly in size at the age of 13 months. However aortic stiffness, measured invasively, was significantly increased only at thirteen months and not at four months in these mice when compared to age-matched wild type controls. This evidence suggests that atherosclerotic lesions develop before arterial stiffness. The exact mechanism by which atherosclerotic lesions cause arterial stiffness is not known. The mechanism may be a direct result of foam cell accumulation in the intima, but secondary responses to inflammation and oxidation provoked by foam cells such as elastin degradation, endothelial dysfunction and medial thickening must all play a role.<sup>99</sup> Young subjects with isolated hypercholesterolaemia have normal or increased arterial compliance

suggesting that the latter mechanisms are more likely to blame,<sup>100</sup> but given the evidence that atherosclerosis and arterial stiffening frequently coexist and that causative mechanisms appear to be similar, the exact relationship remains uncertain.

### ***Diabetes mellitus and metabolic syndrome***

Many studies have found increased arterial stiffness across all age groups in subjects with diabetes and metabolic syndrome. The closest correlation has been observed in obese children with metabolic syndrome.<sup>101</sup> Study populations with metabolic syndrome show increased arterial stiffness in proportion with the degree of metabolic derangement.<sup>102</sup> Increased stiffness is demonstrated in populations with decreased glucose tolerance<sup>103</sup> and insulin resistance.<sup>104</sup> Arterial stiffening, therefore, may be present from the beginning of an insulin resistant state, and before type 2 diabetes develops.

Comparing a population of young patients with type 1 diabetes to healthy age matched volunteers; acute hyperglycaemia had no effect on arterial stiffness in healthy controls but did cause increased stiffness in the diabetic group. Interestingly the baseline arterial stiffness in both populations was similar, suggesting that the development of arterial stiffness in type 1 diabetes is related to exposure to chronic hyperglycaemia.<sup>105</sup>

The chronic state of hyperglycaemia and hyperinsulinaemia stimulates the renin angiotensin aldosterone system (RAAS) and

angiotensin type 1 receptor expression in vascular tissue<sup>106</sup> thus causing wall hypertrophy and fibrosis<sup>107</sup> and promotes non-enzymatic glycation of proteins with subsequent AGE production. Further stiffening is caused by endothelial dysfunction stimulated by high LDL, free fatty acids,<sup>108</sup> endothelin-1, decreased levels of adiponectin<sup>109</sup> and natriuretic peptides.<sup>110</sup>

### ***Hypercholesterolaemia***

Animal studies have shown that hypercholesterolemia caused by cholesterol rich diet provokes an initial decrease in arterial stiffness, followed an increase in stiffness over time which is then reversed by lowering serum cholesterol.<sup>96,111,112</sup>

Data from current research studies suggests a positive correlation between increased arterial stiffness and familial hypercholesterolemia. However this evidence is not conclusive as the studies have used a number of different methods of measuring arterial stiffness and several have small sample sizes. Familial hypercholesterolemia is characterised by high plasma levels of low-density dietary protein, and the prevalence of premature and severe coronary disease. The available data has produced conflicting results in both children and adults. Lehman et al reported increased aortic distensibility in children with familial hypercholesterolaemia but decreased aortic distensibility in adults.<sup>100</sup> However Virkola et al<sup>113</sup> and Aggoun et al<sup>114</sup> both found reduced carotid distensibility in children. Pitsavos et al report decreased aortic

distensibility in adults,<sup>115</sup> and Giannattasio et al report the same when looking at radial artery compliance.<sup>116</sup> In contrast, Toikka et al, looking at carotid and aortic distensibility, found no change in arterial stiffness in a case-control study.<sup>117</sup>

The evidence for an association between raised lipid sub-fractions and increased arterial stiffness in the general population is conflicting, and has been summarised as shown in Table 2. As can be seen, whilst a number of the studies demonstrate a positive correlation between HDL-cholesterol and arterial stiffness,<sup>118-122</sup> and a few demonstrate a negative correlation with LDL cholesterol,<sup>121,123,124</sup> there are also many studies that demonstrate no conclusive relationship.<sup>125-130</sup>

**Table 2:** Summary of relationship between arterial stiffness and dyslipidaemia

Reference	Population	Measures	Main findings
Taquet <sup>125</sup>	429 healthy women	aortic PWV	no independent relationship with cholesterol
Giral <sup>118</sup>	105 hypercholesterolaemic men	aortic PWV	independently positive association with HDL3 cholesterol
Pirro <sup>126</sup>	60 hypercholesterolaemics, 25 controls	aortic PWV	independent negative correlation with LDL cholesterol, disappeared after correction for CRP and waist circumference
Lebrun <sup>127</sup>	385 post-menopausal women	aortic PWV	positive relationship with triglycerides, inverse with HDL cholesterol
Czernichow <sup>128</sup>	917 middle-aged subjects	aortic PWV	no independent association with HDL cholesterol
Wilkinson <sup>119</sup>	68 hypercholesterolaemics, 68 matched controls	augmentation index	augmentation index higher in the hypercholesterolaemic subjects and a positive relationship with LDL cholesterol
Alagona <sup>129</sup>	18 low HDL cholesterol subjects, 18 matched controls	augmentation index	no difference between groups
Dart <sup>130</sup>	868 hypertensives >65yrs	augmentation index; SAC	no relationship with total or HDL cholesterol
Dart <sup>120</sup>	30 hypercholesterolaemics, 24 controls	aortic distensibility ( $\beta$ index)	cholesterol positively related to $\beta$ index
Hopkins <sup>121</sup>	38 healthy adults	aortic distensibility	inverse correlation with total and LDL cholesterol, and positive correlation with HDL cholesterol
Toikka <sup>117</sup>	25 healthy men	aortic and carotid compliance	carotid but not aortic compliance associated with low HDL: total cholesterol ratio; oxidized LDL correlated with carotid compliance
Urbina <sup>131</sup>	516 random subjects	carotid elastic modulus	positive independent association with triglycerides only
Giannattasio <sup>122</sup>	10 controls, 10 hypertensives, 10 hypercholesterolaemics, 10 mixed	radial artery distensibility	reduced distensibility in both hypercholesterolaemic groups
Cameron <sup>123</sup>	20 patients with CAD, 20 controls	SAC	inverse association with LDL cholesterol
Le <sup>124</sup>	223 subjects with CAD risk factors	SAC	inverse correlation with triglycerides and non-HDL cholesterol

**Legend:** PWV=pulse wave velocity; HDL=high density lipoprotein; LDL=low density lipoprotein; SAC=systemic arterial compliance; CAD=coronary artery disease. (after Wilkinson)<sup>132</sup>

### ***Peripheral arterial disease***

Peripheral arterial disease (PAD) describes a degenerative arteriopathy which predominantly affects the distal aorta and vessels supplying the lower limbs. It is characterised by the formation of atherosclerotic plaques, with a varying degree of in situ thrombosis. In addition, fibrosis and calcification of the tunica media is often present. The major symptom of PAD is intermittent claudication which is defined as pain and weakness in the affected limb on exercise, relieved by rest. This symptom, however, is absent in most subjects with PAD.<sup>133</sup>

The ankle brachial pressure index (ABPI) is a useful non-invasive screening tool to look for the presence of PAD in subjects. It is measured using a blood pressure cuff and a hand held Doppler (see Figure 5). In this way the ratio of systolic blood pressure (SBP) at the brachial artery is compared to the SBP in the arteries at the ankle. In a healthy subject, the ABPI is greater or equal to 1.0. It may be greater than 1.0 due to the large amount of reflected pressure waves from the toes merging with the forwards systolic pressure wave only a short distance away at the ankle. In disease, as the arterial lumen narrows, SBP drops distal to the obstruction, and thus the ABPI is found to be less than 1.0. A ratio of less than 0.9 is considered to be abnormal, demonstrating either systolic hypertension or decreased lower limb perfusion. This ratio has been shown to be a reliable predictor of PAD<sup>134</sup> and also coronary artery disease (CAD) and stroke.<sup>135</sup> More recently, high ABPI greater than 1.4 have been shown to be a positive predictor for all cause and



cardiovascular mortality, as this is an indication of much increased arterial rigidity.<sup>136</sup>

**Figure 5:** Measurement of the ankle brachial pressure index



**Legend:** After Wolfe<sup>137</sup>

### **Systemic changes in PAD**

Systolic and diastolic blood pressure has consistently been found to be higher in subjects with PAD than in aged-matched controls, when blood pressure is determined non-invasively.<sup>138</sup> This may be due to the presence of atheroma or hypertrophy of the tunica media. It results in inappropriately elevated pressures when calf pressures are compared with intra-arterial measurements.<sup>139-141</sup> In particular, in the elderly, it

appears as though non-invasive measurement overestimates diastolic blood pressure (DBP) compared to SBP.<sup>142</sup> Intra-arterial studies of brachial artery pressure in hospitalised patients with PAD, compared with age and sex matched controls, suggest that DBP is relatively unchanged compared with SBP in which a sustained and significant rise was found. This finding was maintained even when subjects with PAD were compared with normal subjects with the same mean arterial pressure (MAP).<sup>143,144</sup> It is likely that this change in pulse pressure (PP) in the brachial artery is also found in the aorta, as the age-related increase in PP centrally is probably greater than that found peripherally.<sup>145</sup> Given that subjects with PAD have a relatively normal cardiac output and systemic vascular resistance.<sup>143,144</sup> The observed change in PP appears to be mainly due to changes in arterial stiffness and wave reflection. *In vitro* studies have demonstrated that reduced large arterial elasticity can cause an elevated SBP whilst leaving DBP unchanged or perhaps even lowered, as found in subjects with PAD.<sup>146,147</sup>

It is likely that the increased arterial stiffness observed in patients with PAD affects the whole arterial tree. This observation is made on the basis of increased PWV found in subjects with PAD when compared to controls with similar MAP,<sup>148,149</sup> as well as increased femoral and carotid arterial stiffness in subjects with PAD.<sup>150,151</sup> The other important factor to consider is wave reflection. In healthy subjects, the distal abdominal aorta is recognised as an important site for wave reflection.<sup>145,146</sup> This site may take on increased importance in subjects with PAD, as the smaller

luminal diameter and increased arterial stiffness found in an atherosclerotic distal aorta would cause the reflected pressure waves to travel more quickly, and thus the forwards and backwards moving pressure waves would superimpose during systole causing an increase in SBP. There is limited evidence to support this theory in subjects who had undergone traumatic amputation of the lower limbs and who later in life demonstrate a high incidence of systolic hypertension.<sup>152</sup>

### **Role of endothelial dysfunction in PAD**

An increasing body of evidence exists that suggests that increased arterial stiffness with PAD is due to more than just structural arterial wall change. This evidence of endothelial dysfunction is demonstrated by impaired flow mediated dilatation in the brachial artery,<sup>153</sup> elevated plasma concentrations of markers of endothelial dysfunction,<sup>154</sup> and a higher increase in brachial SBP after isotonic saline infusion than in matched control populations.<sup>155</sup> In contrast arterial stiffness was shown to be improved in subjects with PAD after exercise,<sup>156</sup> L-arginine administration,<sup>157</sup> autologous bone marrow transfusion,<sup>158</sup> nitrate administration,<sup>146,152</sup> and Ramipril, an angiotensin converting enzyme inhibitor (ACE-I).<sup>159</sup>

### **Effect of arterial stiffness on haemodynamics in PAD**

There is a strong positive correlation between systemic blood pressure and calf blood flow at rest in subjects with PAD, a relationship not found in healthy subjects,<sup>160</sup> with studies demonstrating that calf blood flow in

subjects with PAD remains normal at rest.<sup>161,162</sup> These findings suggest that there may be regulation of systemic blood pressure in order to promote lower limb perfusion in the presence of PAD.

PP also has a positive correlation with calf blood flow at rest, and this relationship is maintained for PP during exercise, unlike MAP. As a result, the higher PP is at rest, the greater is the reduction in walking distance.<sup>160</sup> This suggests that increases in baseline arterial stiffness have an important role in the symptomatic severity of subjects with intermittent claudication.<sup>133</sup>

### ***Cerebrovascular disease***

Cross-sectional studies demonstrate good correlation between markers of arterial stiffness and risk factors for stroke.<sup>163,164</sup> Increased aortic stiffness has been demonstrated in subjects with cerebrovascular disease (CVD) who have suffered a cerebrovascular accident (CVA).<sup>95</sup> Longitudinal studies have demonstrated that increased arterial stiffness is an independent risk factor for cerebrovascular events in a population with primary hypertension<sup>165</sup> and a separate population of healthy elderly subjects.<sup>166</sup>

### ***Coronary artery disease***

As with other clinical manifestations of atherosclerosis, the available evidence suggests that arterial stiffness may be a cause of CAD, a consequence and also an exacerbation.<sup>167</sup> There is good evidence that arterial stiffness is associated with CAD,<sup>94,168-173</sup> myocardial

ischaemia<sup>167,174</sup> and increased mortality from heart disease.<sup>175</sup> Positive correlation between pulse pressure and CAD has also been demonstrated in a longitudinal study of a population of subjects with hypertension.<sup>175</sup> The mechanisms by which arterial stiffness may cause CAD are not clear, but increased arterial stiffness is likely to cause systolic hypertension, which in turn would cause increased cardiac afterload and left ventricular hypertrophy. Given that most cardiac perfusion occurs during diastole, decreased diastolic pressure would reduce this and thus, in conjunction with an increased pulse pressure, demand would increase and supply would diminish.<sup>176</sup> Animal studies have demonstrated that cardiac energy consumption and pulse pressure increase when cardiac ejection is into a stiffer aortic conduit.<sup>177</sup> Furthermore cardiac perfusion in a heart with a deliberately created coronary artery stenosis is much worse when ejection is into a stiffer aorta.<sup>178</sup> Leung et al have provided some corroborative clinical evidence for these findings by demonstrating that both coronary blood flow and subsequent improvement in blood flow was lower in subjects with higher aortic stiffness as measured by PWV when undergoing percutaneous coronary reperfusion.<sup>174</sup>

### ***Renal disease***

Access to dialysis in end stage renal disease (ESRD) is no longer restricted. As a result, patients with ESRD are living longer and are dying from cardiovascular complications of renal failure rather than the disease

itself. The mortality in this population is in the order of 10% per annum,<sup>179</sup> and arterial stiffening is a strong independent predictor of this.<sup>180</sup> The mechanisms implicated for the observed accelerated arterial degeneration are:

- increased IMT secondary to increased wall stress from hypertension,<sup>30</sup>
- increased ECM collagen content and VSMC proliferation,<sup>30</sup>
- decreased collagen elasticity due to AGE formation and reaction with methylglyoxal and other reactive carbonyl compounds which are found in increased amounts in uraemia<sup>181</sup> and
- calcification of the media.<sup>182</sup>

In subjects with a renal transplant, increased stiffness is strongly correlated with the length of time that these subjects received haemodialysis.<sup>183</sup>

## Conclusions

Whilst it is widely accepted that arterial stiffness increases with age, there is no reliable animal model available for research. Most studies are cross-sectional in design and may therefore only include fit survivors by default. There is now a large body of evidence to support a relationship between increased arterial stiffness, cardiovascular disease and disease states known to promote cardiovascular disease. In particular subjects with PAD (diagnosed by reduced ABPI) have been shown to have increased arterial stiffness and be at high risk of future cardiovascular events.

## MODULATION OF ARTERIAL STIFFNESS

*Given the association of increased arterial stiffness with increased prevalence of cardiovascular disease, the assumption has been made that decreased arterial stiffness is a worthwhile therapeutic goal. Currently there is little published evidence to support this. Methods to decrease arterial stiffness are based on modifications of diet, lifestyle and the addition of pharmacological agents, usually anti-hypertensives or statins. These methods can be successful in reducing arterial stiffness, but evidence that this effect translates into clinical benefit is lacking.*

## Introduction

Perhaps unsurprisingly, most methods of reducing arterial stiffness have been found or have been investigated as a by product of treating cardiovascular disease and its risk factors. As will be seen, these methods can be categorised into modification of lifestyle or addition of a pharmacological agent.

Based on the evidence that subjects with many chronic diseases have increased arterial stiffness when compared to a healthy control population, and that subjects such as athletes or the physically active elderly have decreased arterial stiffness when compared to a normally active control population,<sup>184,185</sup> the presumption has been made that reducing arterial stiffness is a worthwhile therapeutic goal. However there is little evidence to support this, as randomised clinical trials with both the aim of decreasing arterial stiffness and the appropriate outcome measures (such as morbidity or mortality) are yet to be reported in the literature. The only study of this sort revealed by literature search was carried out in patients with end-stage renal disease, and thus is not applicable to the general population.<sup>186</sup> This paucity of research is perhaps not surprising given not only the controversy surrounding measurement of arterial stiffness but also the lack of consensus on desired therapeutic goal in terms of degree of reduction of arterial stiffness. If this becomes clear, together with a clearer picture of the contribution to cardiovascular risk that increased arterial stiffness makes,



then targeted therapy may become part of best medical therapy to reduce cardiovascular risk on the basis of future urgently required clinical trials.

## **Modification of lifestyle**

The most successful lifestyle modification appears to be endurance exercise. In healthy individuals, without any evidence of cardiovascular disease but who have increased arterial stiffness secondary to ageing, the increase in arterial stiffness was found to be less in those who participated in regular exercise.<sup>184</sup> It was also found that a three-month programme of aerobic training decreased levels of carotid artery stiffness in middle-aged men with known arterial stiffness to similar levels of arterial stiffness in endurance trained men of the same age.<sup>187</sup> These benefits appeared to continue after exercise training has ceased,<sup>188</sup> and appear to be secondary to mechanical stimulation of the endothelium resulting in enhanced nitric oxide production together with decreased release of neurohumoral vasoconstrictors and efferent sympathetic tone.<sup>189</sup> However no decrease in arterial stiffness was found in trials of aerobic exercise in subjects with systolic hypertension<sup>190</sup> or subjects who were very elderly.<sup>191</sup> Anaerobic training, such as weightlifting, has been shown to increase arterial stiffness.<sup>192</sup> Weight loss in healthy men has been shown to reduce arterial stiffness but this finding was not maintained when adjusted for decrease in blood pressure.<sup>193</sup>

Reduction of salt intake decreases arterial stiffness in the short and long-term, independent of the effect on blood pressure.<sup>194,195</sup> Salt

exposure induces structural changes in the vascular wall that have been identified in rats fed on a high salt diet.<sup>196</sup> This may explain the accelerated age related arterial stiffening found in subjects who have a high salt intake.<sup>88</sup>

Alcohol consumption similarly lowers arterial stiffness independent of blood pressure,<sup>197,198</sup> but this relationship is weaker when adjusted for HDL cholesterol. HDL cholesterol promotes reverse cholesterol transport and is increased with exposure to alcohol, thus suggesting that the effect that alcohol exposure has on arterial stiffening is mediated by increased levels of HDL cholesterol.<sup>179</sup> Moderate alcohol exposure in healthy males has additionally been found to increase cellular cholesterol efflux.<sup>199</sup>

Other dietary additions have also been shown to decrease arterial stiffness, notably isoflavones,<sup>200,201</sup> fish oil,<sup>202</sup> garlic,<sup>203</sup> and flax seed oil.<sup>204</sup>

## **Pharmacological modulation**

As previously discussed, most pharmacological agents that have been shown to decrease arterial stiffness are anti-hypertensives, which cause this effect indirectly as a result of reduction in MAP or PP. Some antihypertensive appear to have little or no effect (diuretics, B-blockers, dihydralazine). This section focuses on medications which appear to have an effect on the structure and function of the arterial wall, and (with the exception of nitrates) which appear to decrease arterial stiffness independent of effect on blood pressure.

## ***Statins***

Statin is the common term for 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors. This class of drug has progressed from use merely in cholesterol lowering therapy to being viewed as the main anti-atherosclerotic agent with pleiotropic effects well beyond the management of lipoproteins.

As the name implies, the main action of statins are to block the rate-limiting step in cholesterol synthesis by inhibiting the enzyme HMG-CoA reductase. Thus at therapeutic levels, both serum total and LDL cholesterol levels are reduced. The evidence is clear that this is beneficial in terms of both primary and secondary prevention of cardiovascular events in subjects with high hypercholesterolaemia.<sup>205</sup> However the preventative effect of statins has also been shown in subjects without hypercholesterolaemia,<sup>206</sup> thus suggesting that statins may be beneficial in other ways. These mechanisms are summarised in Table 3.

**Table 3:** Summary of the pleiotropic effects of Statins

effect	specific details
<b>anti-thrombotic</b>	reduction in PAI-1 increase in t-PA increase in NOS expression and activity reduction in tissue factor expression reduction in fibrinogen decreased platelet aggregation increase in thrombomodulin
<b>endothelial cell biology</b>	enhanced eNOS expression and activity reduction in genes for leucocyte adhesion anti-inflammatory signals reduction in endothelial inability increase endothelial growth increase in endothelial progenitor cells/angiogenesis reduction in pre-pro-ET-1 expression
<b>vascular smooth muscle cells</b>	reduction in VSMC growth and migration decrease in expression of AT1 receptor in response to an injurious stimuli
<b>miscellaneous</b>	Reduction in generation of oxidant species increase in gene expression of antioxidants decrease in fibroblast growth and collagen formation inhibition of LDL oxidation and ox-LDL uptake inhibition of mitogen-stimulated T-and B-lymphocyte proliferation inhibition of expression of class two MHC blockade of the effects of angiotensin II inhibitor of natural killer cell cytotoxicity stimulation of bone formation

**Legend:** PAI-1=plasminogen activator inhibitor type 1; t-PA=tissue plasminogen activator type 1; NOS=nitric oxide synthase; eNOS=endothelial nitric oxide synthase; pre-pro-ET-1=pre-pro-endothelin-1; VSMC=vascular smooth muscle cell; AT1=angiotensin type 1; LDL=low density lipoprotein; ox-LDL= oxidised low density lipoprotein; MHC=major histocompatibility complex. (after Sinha)<sup>207</sup>

There are few studies looking at the direct effect of statins on arterial stiffness. These are summarised in Table 4. The data is difficult to interpret due to the use of different statins in separate studies. In addition the method used to measure arterial stiffness varies from study to study. The overriding implication from this research appears to be that long term use of atorvastatin and fluvastatin decreases arterial stiffness in a variety of study populations with or without hypertension, and that this finding is independent of effect on systemic blood pressure.<sup>208-210</sup> Interestingly the short term results of Statin exposure suggest a transient rise in arterial stiffness, hypothesised to be due to the initial reduction in lipid vascular content.<sup>211</sup>

The most important mechanisms to explain these findings would appear to be increased activity of endothelial nitric oxide synthase and thus increased production of nitric oxide in vascular endothelial cells.<sup>212</sup> As previously discussed NO is a potent vasodilator and platelet inhibitor. The direct effect of HMG-CoA reductase inhibition is to prevent the conversion of HMG CoA into mevalonate. Mevalonate is the precursor of cholesterol and also isoprenoids which are involved in the lipid modification of regulatory proteins.<sup>213</sup> Thus down-regulation of isoprenoids may alter cell signalling, proliferation and intracellular trafficking. In addition statins may have immunomodulatory effects,<sup>214</sup> and may promote inhibition of LOX-1, a receptor for oxidised LDL that is known to be up regulated in atherosclerotic lesions, and which promotes the release of matrix metalloproteinases.<sup>215</sup> Decreased quantities of

plasminogen activator inhibitor type 1 (PAI-1) and increased quantities of tissue plasminogen activator (t-PA) have been noted in endothelial cells treated with statins. T-PA is important in the control of tissue remodelling, fibrinolysis and atherosclerosis and is inhibited by PAI-1.<sup>216,217</sup>

**Table 4:** Summary of effect of statins on arterial stiffness

Author	Study population	Study drug(s)	stiffness measurement	Result
<b>Ichihara<sup>208</sup></b>	85 subjects with hyperlipidaemia and hypertension	pravastatin fluvastatin simvastatin lower than normal therapeutic doses	aortic PWV	PWV decreased transiently in simvastatin group, significantly in fluvastatin group, not with pravastatin after follow up for 1 year
<b>Raison<sup>211</sup></b>	23 subjects with hypertension and hypercholesterolaemia	atorvastatin or placebo	aortic PWV	no change in BP but increased PWV after 3 months in atorvastatin group
<b>Kontopoulos<sup>209</sup></b>	36 subjects with hypercholesterolaemia but no hypertension or diabetes. 18 with CAD and 18 without CAD	atorvastatin	transthoracic echocardiography	aortic stiffness significantly reduced after 2 years
<b>Ferrier<sup>210</sup></b>	22 subjects, normolipidaemic with isolated systolic hypertension	atorvastatin	carotid applanation tonometry and Doppler velocimetry of the ascending aorta	systemic arterial compliance significantly increased in subjects taking atorvastatin after 3 months

## ***Nitrates***

Several trials have studied the effect of nitrates on arterial stiffness. The main effect appears to be a reduction in pulse pressure brought about by a decrease in SBP but not in DPB. Duchier et al and Starmans-Kool et al looked at the effect of chronic isosorbide dinitrate (ISDN) administration in double-blind randomised placebo controlled studies.<sup>218,219</sup> They both found a decrease in SBP with ISDN when compared to placebo, but no decrease in DBP. This suggests that the main effect of nitrates is on smooth muscle tone in the larger muscular arteries as opposed to an effect on the smaller high resistance vessels which would be manifest by a decrease in DBP. However the long term effect of nitrate administration is hampered by the development of tolerance.

Sinitrodil (an NO donor) administration, caused a greater increase in brachial artery compliance but a lesser decrease in total peripheral resistance than ISDN in healthy subjects suggesting that it may be possible to develop more selective drugs that act on a large arterial stiffness.<sup>220</sup>

## ***Renin angiotensin aldosterone system***

The main drug class acting on the renin angiotensin aldosterone system (RAAS) are Angiotensin Converting Enzyme Inhibitors (ACE-Is). As well as being recognised as effective anti-hypertensive agents, they are of great interest in the treatment of atherosclerosis as they have been shown to protect against secondary coronary events in subjects with

cardiovascular disease.<sup>221</sup> This effect was found to be independent of blood pressure reduction, suggesting that this may be due to vessel wall remodelling. Animal models have demonstrated that activation of the RAS causes expansion of the extra cellular matrix (ECM) and fibrosis.<sup>196,222</sup> This fibrosis is characterised by the presence of inflammatory cells and activation of tissue response to injury pathways.<sup>223</sup> The remodelling effect of ACE-Is in mouse, pig, rabbit and monkey models of atherosclerotic disease has been demonstrated showing reduction in size of atherosclerotic lesions.<sup>224-229</sup> In addition improvement of endothelial dysfunction was shown in subjects with coronary artery disease after ACE-I administration.<sup>230,231</sup>

### ***Aldosterone antagonists***

Rat model experiments have suggested that aldosterone infusion causes increased rigidity of the aortic wall in the presence of a high salt diet, a phenomenon that was reversed when epleronone, an aldosterone antagonist, was administered.<sup>232</sup> Using immunohistochemical staining, Lombes et al found a higher density of mineralcorticoid receptors in the proximal aorta with the density decreasing with arterial diameter.<sup>233</sup> This provides a potential explanation for the way in which aldosterone agonists reduce arterial stiffness. However despite the fact that increased plasma aldosterone and increased arterial stiffness has been found in untreated hypertensives,<sup>234</sup> a study treating hypertensive subjects with



spironolactone did not demonstrate any reduction in arterial stiffness, perhaps because subjects were only given the drug for two weeks.<sup>235</sup>

## **Conclusions**

Modulation of arterial stiffness has been shown to be successfully managed via changes in lifestyle, in terms of diet and exercise. Attention to pharmacological modulation has focused around antihypertensive drugs and statins. Certain statins (atorvastatin and fluvastatin) have been shown to decrease arterial stiffness with chronic use, perhaps as a result of their pleiotropic effects. ACE-Is have also been shown to decrease arterial stiffness independently of reduction in blood pressure. There is limited evidence that this may be due to vessel wall remodelling. Other anti-hypertensives probably manifest a reduction in arterial stiffness as a result of decrease in blood pressure.

## 6

# OVERVIEW OF A TISSUE DOPPLER IMAGING BASED METHOD TO CALCULATE INDICES OF ARTERIAL STIFFNESS

*The TDI based system uses Doppler scanning and dedicated software to identify and capture information about arterial wall motion. This is performed by capturing cine-loops of 5 seconds duration and then transferring them offline to a personal computer for analysis by accompanying software. This allows calculation of the arterial distension waveform. Together with measurement of intima-media thickness and brachial blood pressure it is then possible to calculate indices of arterial stiffness.*

## Introduction

The basic parameters that an ultrasound system can measure are distance, time and velocity. All other parameters are inferred and historically very time consuming to calculate, as well as having quality control issues in terms of inter and intra operator reproducibility. The main advantage of ultrasound when compared to other imaging modalities remains that it is the only method able to generate real-time images without the use of ionising radiation.

The tissue Doppler Imaging (TDI) system developed by Philips ATL applies broadband Doppler processing and dedicated scanning sequences which are designed to accommodate the low tissue motion velocity of arterial walls ( $\text{max } 2 \text{ cms}^{-1}$ ) and also the high frame rate (40 Hz) required to accurately sample arterial wall motion (AWM) over the cardiac cycle. It has been shown using a moving platform phantom that the TDI technique has a resolvable displacement of  $22 \text{ }\mu\text{m}$  and a measurement accuracy of 8% using a physiological wall motion with a peak displacement of  $689 \text{ }\mu\text{m}$ .<sup>236</sup>

The known limitations to this system are that the software will not accurately identify the arterial walls in the following circumstances:

- the insonated vessel has a lumen of less than 3 mm,
- the vessel has a branch in the area to be interrogated, and
- vessel is more than 7 cm below the skin surface.<sup>237</sup>

This last limitation is due to the fact that TDI software will only run using data collected from an L 12/5 linear array of the HDI 5000 ultrasound imaging system (Philips Medical Systems, Bothell, WA, USA), which has a maximum tissue penetration of up to 7cm.

A number of other factors have been found to affect AWM measurement precision when investigated using an arterial phantom; vessel depth (variation  $7\pm3\%$ ), misalignment of beam-vessel angle from  $90^\circ$  ( $22\pm3\%$ ), degree of scan plane coincidence with the central vessel axis ( $34\pm2\%$ ), and transducer pressure on the insonated vessel ( $20\pm3\%$ ).<sup>238</sup>

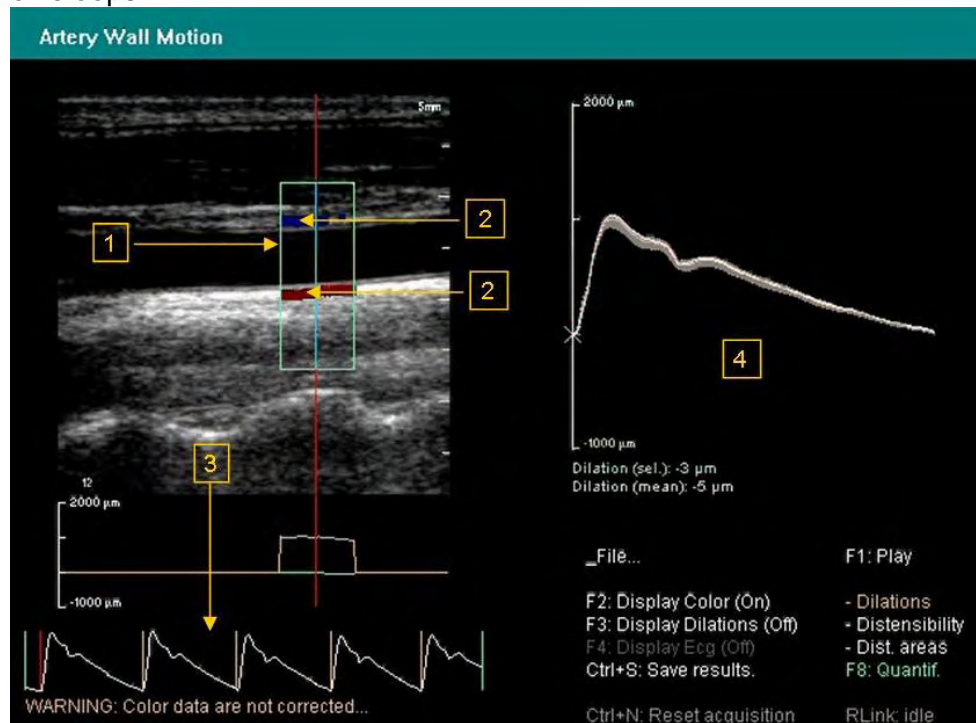
## **Acquisition of scan data**

The common carotid artery (CCA) approximately 3 cm proximal to the carotid bulb, was imaged longitudinally using the L12/5 linear array of an HDI 5000 ultrasound imaging system (Philips Medical Systems, Bothell, USA). Scan-plane alignment was performed using B-mode imaging to ensure that echoes from the intima-media layers were clearly visible. Tissue Doppler imaging (TDI) was enabled and real-time images were collected of a 2 cm segment of the CCA over at least three cardiac cycles. This was saved to disc as a cineloop and transferred off-line for analysis via an Ethernet connection to a laptop computer.

## Offline analysis of arterial distension waveform data

After transfer of the raw cineloop to the PC, a commercial software analysis package (HDI-Lab, Philips Medical Systems, Bothell, WA, USA) was used to obtain wall distension waveforms from the TDI data (Figure 6).

**Figure 6:** Screenshot of the AWM software used to analyse captured cineloops.

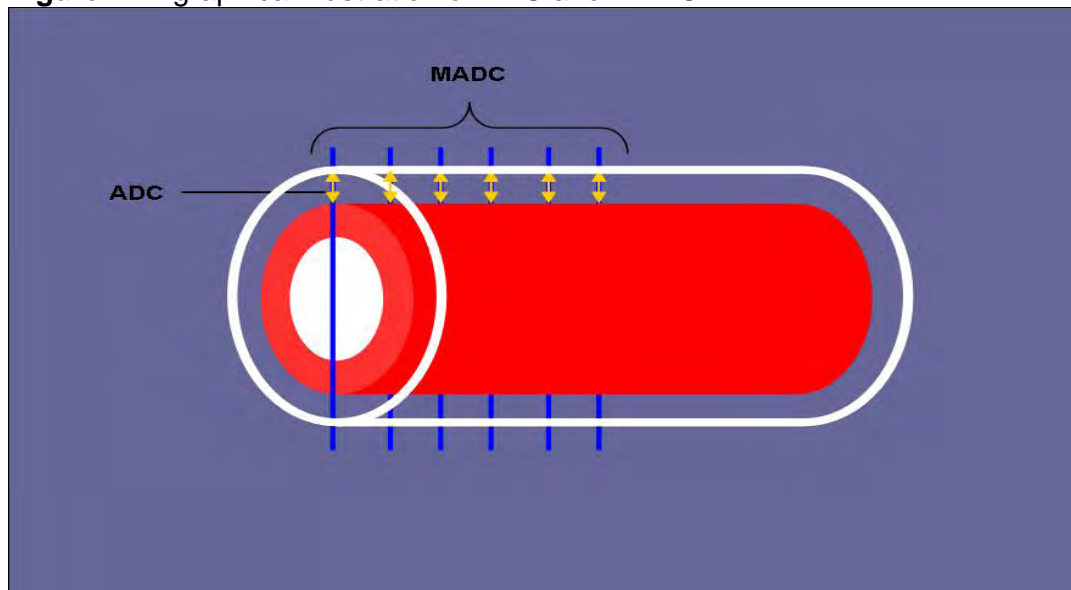


**Legend:** 1 moveable box this can be changed in size in order to direct the program to interrogate the region of interest (ROI); 2 colour information that indicates direction of movement of the arterial wall over time; 3 ADW trace shown graphically for entire length of cineloop; 4 detailed graph of ADW for each cardiac cycle. The thick grey line is made up of a plot of all 50 lines of information. As movement is so homogeneous in this sample, it appears as one thick grey line. The white line represents the mean of all 50 grey lines (MADC).

The core feature of this technique is that TDI data provides information on wall velocity as a function of time. Wall motion is then

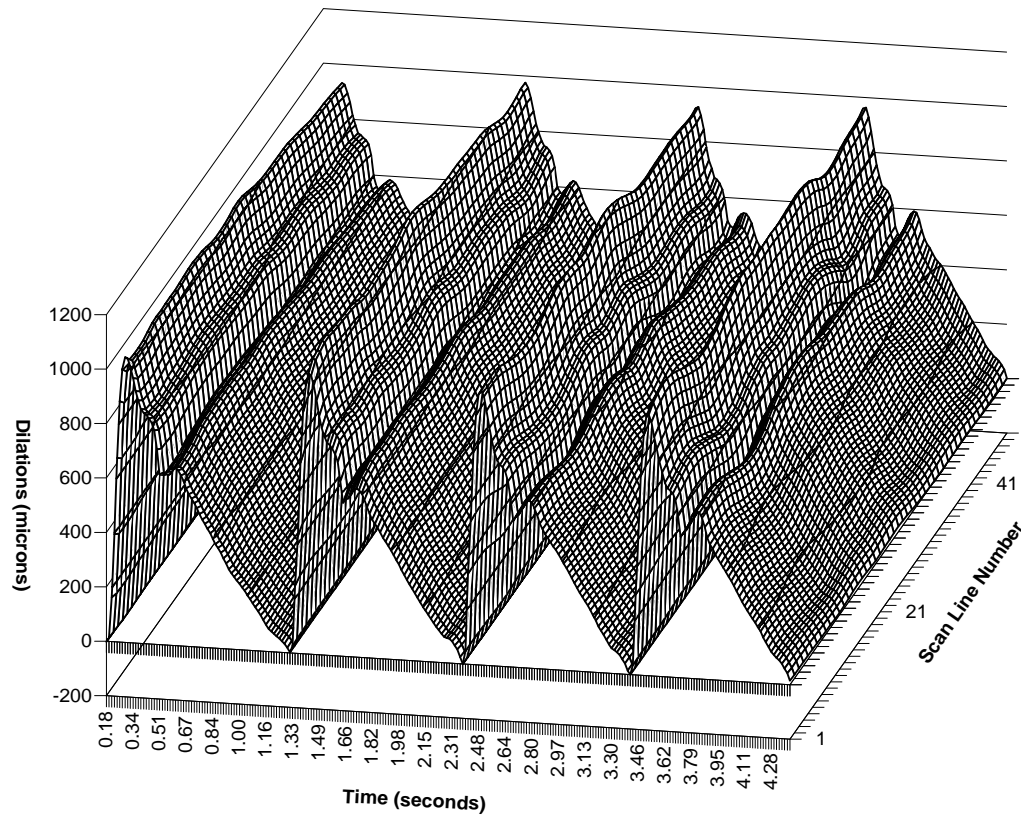
calculated by integration of velocity with respect to time. The system interrogates wall motion throughout all recorded cardiac cycles in the cine loop. This interrogation is performed for each scan-line of the TDI image and is recorded as change from baseline (in micrometers), thus providing some 50 measurements of the distension-time waveform over a 2 cm length of artery (see figure 7). The arterial diameter change (ADC) for each line can be pooled to allow calculation of the mean arterial diameter change (MADC) for use in calculation of indices of arterial stiffness. Alternatively the ADC for each scan line can be plotted to provide the arterial distension waveform (ADW, Figure 8).

**Figure 7:** A graphical illustration of ADC and MADC.



**Legend:** The system records wall motion of the artery as change from baseline (yellow arrowed lines in figure) over the cardiac cycle along 50 scan lines (blue lines in figure). This can then be plotted as a distension waveform (as demonstrated in Figure 8) or pooled to calculate mean arterial diameter change to be used to calculate indices of arterial stiffness.

**Figure 8:** A graphical representation of arterial distension waveform



**Legend:** Dilations = how far in microns arterial wall moved apart during cardiac cycle; Scan line number= virtual line down which software calculated arterial wall dilatation (48 line in total); Time = total length in time of scan and scale over which arterial dilatation occurred. This data was captured over 4 cardiac cycles from a segment of common carotid artery measuring approximately 2cm.

This data can then be exported as a Microsoft Excel file from the software, thus allowing numerical interpretation of the waveform. Each Excel file from each scan contains approximately 20000 points of information. These data were summarised to provide the following information. The MADC was calculated from all lines in all cardiac cycles in which the ADC was measured by obtaining the maximum excursion for each line and then taking the mean of all these values. This process was automated using a Macro in Microsoft Excel to enable efficient extraction

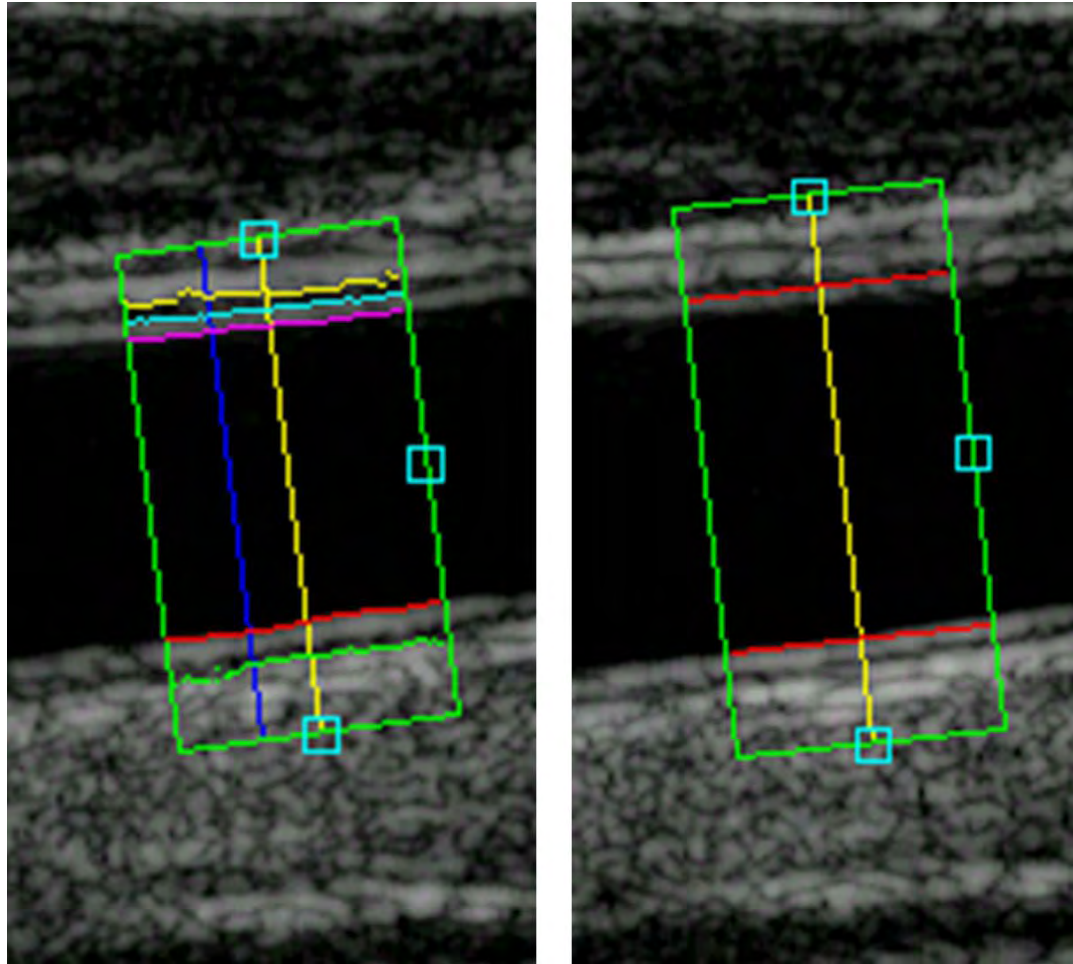
of the required value from the raw data and has been reproduced in Appendix 2.

## **Calculation of IMT and arterial diameter**

IMT and arterial diameter was calculated from the same segment of CCA in the same cineloop transferred to the personal computer using HDI Lab, using commercial software from Philips ATL, (IMT plug-in, LDOT plug in HDI-Lab, Philips Medical Systems, Bothell, WA, USA) that interrogates all digital frames within the cineloop in order to calculate mean IMT and mean diameter via B. mode ultrasound. HDILab uses an edge detection algorithm in order to identify the start and finish of both near and far wall intima media complex in a defined region of interest within the cineloop (Figure 9). This data is then presented as mean IMT for the near and far wall of the artery. Mean IMT for the far wall in all frames across all cardiac cycles captured in the cineloop was used to calculate indices, in view of published advice that IMT changes across the cardiac cycle and that measurement of IMT in the far wall as opposed to the near wall is more accurate.<sup>239</sup>



**Figure 9:** A screenshot of HDI Lab calculating IMT and diameter for both the near and far arterial wall.



**Legend:** green box = defined area of interest. Horizontal yellow line =near wall adventitia-media interface; blue line = near wall intima media interface; purple line = near wall intima lumen interface; red line = lumen intima interface; horizontal green line = far wall media adventitia interface.

## Blood pressure measurement

Three blood pressure measurements in the right brachial artery using a Critikon automated blood pressure manometer (GE Healthcare, Bucks, UK) were recorded whilst the patient remained supine, at the same time as AWM data was collected. One measurement was taken before scan

acquisition; one whilst the data were transferred off-line for analysis and one after data capture was completed. Whilst these blood pressure measurements were taken at the same sitting as AWM data was captured, the measurements were not synchronous. The mean change in blood pressure ( $\Delta p$ ) of the three measurements was used to calculate indices of arterial stiffness as appropriate.

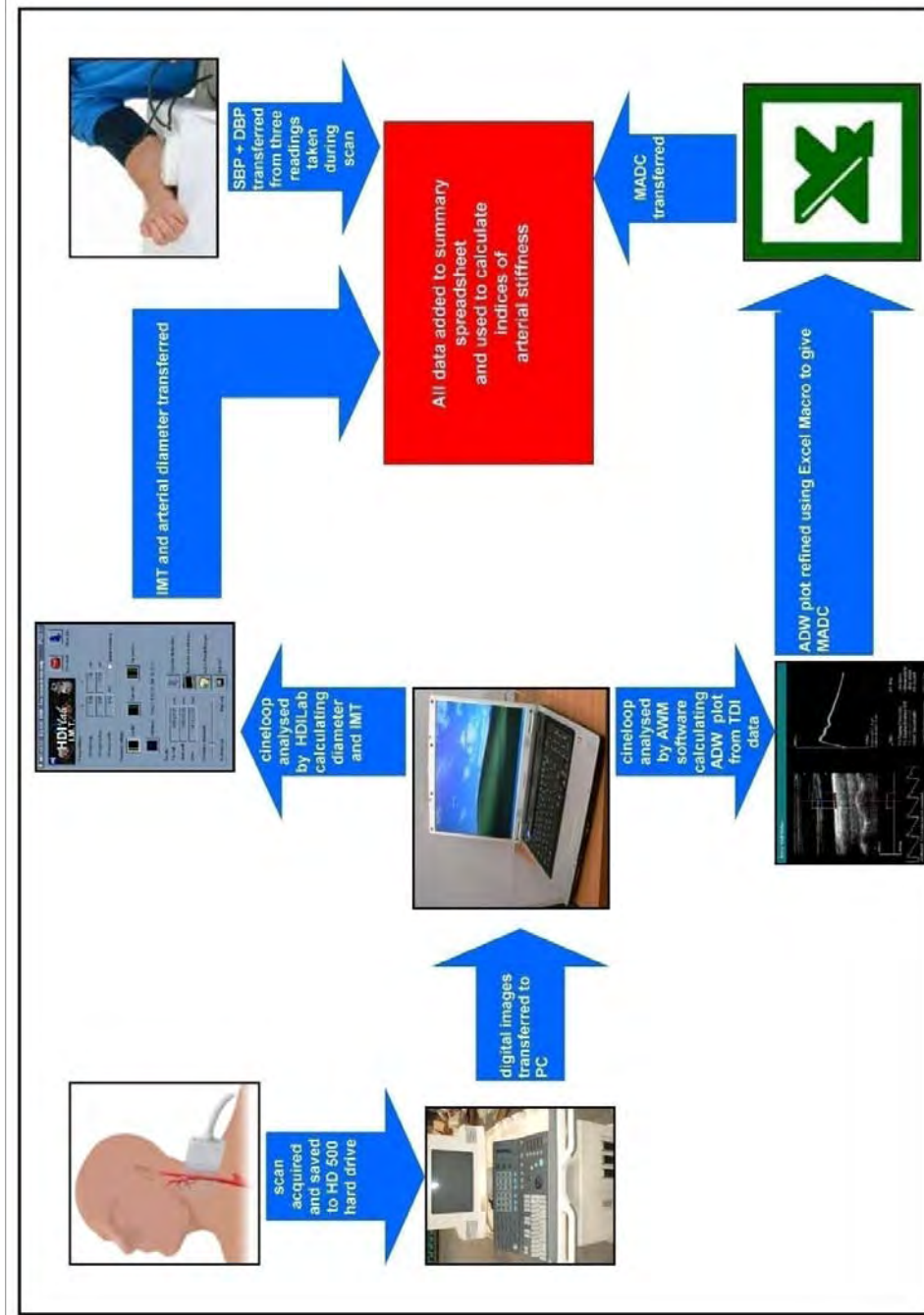
## **Calculation of ankle brachial pressure indices**

In certain parts of the study, ABPIs were recorded using the following method. A manual sphygmomanometer was placed around the mid calf of each leg. The posterior tibial, anterior tibial and perforating peroneal arteries were insonated with a hand held Doppler (Mini Dopplex<sup>®</sup>, Huntleigh Diagnostics, Cardiff, UK). The cuff was inflated until the signal in the insonated artery disappeared, indicating occlusion. The highest pressure at the ankle recorded in any artery on cuff deflation was divided by the highest systolic brachial pressure to give the ABPI for each leg.

## **Conclusions**

The entire system used to collect the data required to calculate indices of arterial stiffness using the distension waveform from the common carotid artery and blood pressure from the brachial artery is summarised in Figure 10.

**Figure 10:** Schematic summary of entire system used to collect data required to calculate indices of arterial stiffness



## INVESTIGATING AND CONTROLLING SCAN VARIABILITY

*The TDI based system to measure arterial stiffness has only been used in a lab based setting. The aim of this study was to use the system to capture data from healthy volunteers in order to develop a basic methodology for validation in a reproducibility study.*

*100 subjects who were either patients or staff at Heartlands Hospital were recruited into the study. Both CCAs in each subject were insonated and cineloops collected from them, together with three blood pressure readings from the right brachial artery. These cineloops were then analysed offline to provide the ADW and subsequently the MADC.*

*Initial data capture was unreliable. Repeated scan acquisitions from the same subject demonstrated that reliable data capture was achieved if correct scan plane alignment was maintained so that the intima-media complex was uniform and fully visible throughout the whole of the ROI within the cineloop. Analysis of the between cycle coefficient of variation, together with evidence from a phantom model suggested that the following requirements were necessary for reliable data capture; diameter greater than 3 mm; no side branches; ensure subject is rested and comfortable on couch prior to scan acquisition for 5 minutes; exclude subjects with breathing difficulties; exclude subjects with cardiac arrhythmias; ensure scan plane alignment in ROI.*

## **Introduction**

The TDI system has never been used on a large number of patients, nor clinically validated. A research group from the Department of Medical Physics at the University of Edinburgh has extensively bench tested this system and found that it is a reliable technique that accurately measures arterial wall displacement when investigated using an arterial phantom.<sup>238</sup>

However, it is not fully known what the limitations of the TDI system are when attempting to calculate indices of arterial stiffness in human subjects. Initially therefore, a variability study was carried out to investigate between cardiac cycle variability of measurement in cineloops captured from the CCAs of subjects in order to develop a reliable method for data collection.

The hypothesis was that the TDI system should allow collection of AWM data from the carotid arteries of human subjects with acceptable and low variability.

## **Method**

### ***Study design***

All subjects recruited into the study provided fully informed written consent. Ethical approval was granted by Birmingham East District Research and Ethics Committee. All subjects completed a questionnaire regarding past medical history and drug evidence.

100 subjects who were either patients or staff at Heartlands Hospital were recruited into the study. Both CCAs in each subject were insonated and cineloops collected from them, together with three blood pressure readings from the right brachial artery. These cineloops were then analysed offline to provide the ADW and subsequently the MADC.

### ***Calculation of variability***

The between cycle coefficient of variation (BCCOV) of the MADC for each cardiac cycle was calculated as it was believed that problems with scan acquisition would be highlighted by disparity in the ADC between each cardiac cycle within a cineloop. The BCCOV was defined as the standard deviation divided by the mean times 100 for the MADCs of each cardiac cycle within each cineloop. All acquired scans were then inspected manually with attention paid to those scans with high or low BCCOV.

## **Results**

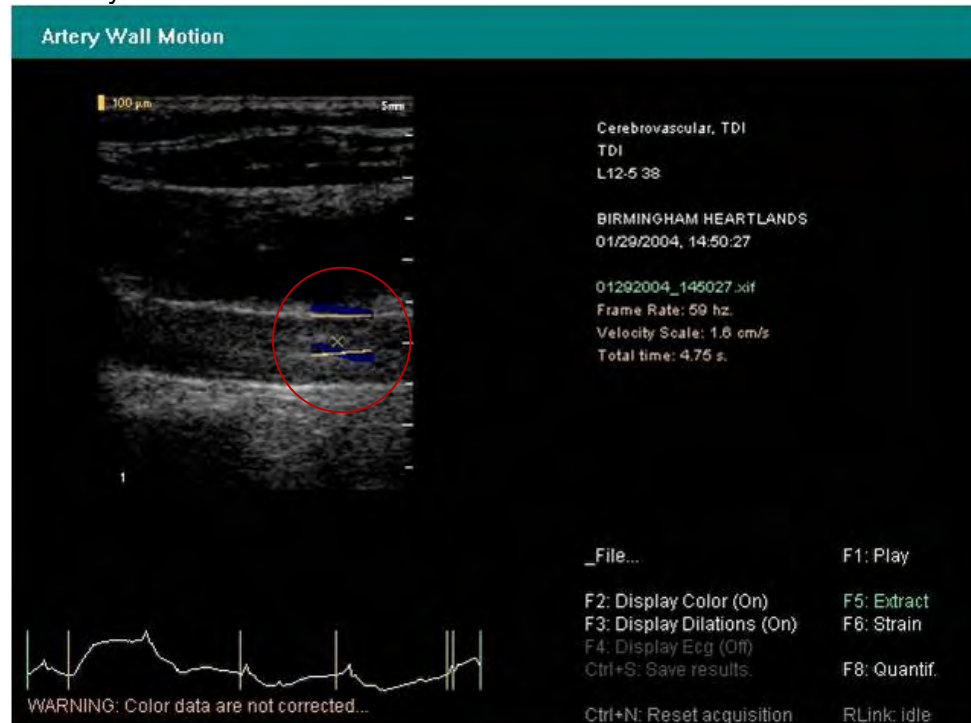
200 hundred scans were collected from 100 subjects. After inspection and analysis, the following errors were found.

### ***Misidentification of the arterial walls***

Initial inspection demonstrated that in 70 cineloops (out of 200), the AWM system had miss-identified the arterial wall (usually the far wall). It was thought that this may relate to greyscale levels within the cineloop as it

was recorded or to artefact within the lumen of the insonated artery (Figure 11).

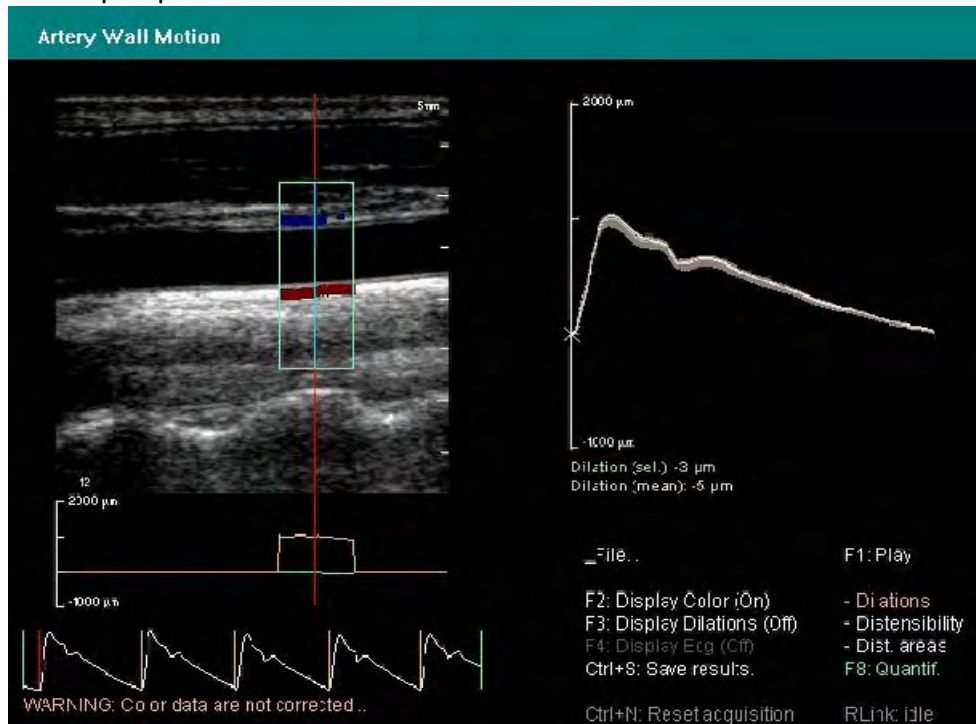
**Figure 11:** Scan acquisition of miss-identification of arterial walls by AWM system



**Legend:** poor scan plane alignment caused part of the side wall of the artery to be miss-identified as the far wall, highlighted within the red circle.

However repeated scan acquisitions from the same subject demonstrated that these issues were resolved if correct scan plane alignment was achieved so that the intima-media complex was uniform and fully visible throughout the whole of the ROI within the cineloop. The AWM System was then able to consistently identify the arterial walls correctly and analyse the cineloop to provide a complete data set of the ADW across all cardiac cycles, as is demonstrated in Figure 12.

**Figure 12:** Screenshot of successful scan plane alignment during cineloop capture.



**Legend:** The Intima-media complex is uniform in the ROI, suggesting ideal scan plane alignment bisecting the diameter of the artery and resulting in correct identification of both arterial walls.

### Extra Arterial Movement

Extra arterial movement was noted when the cineloops were viewed off line in a number of scans. Two extreme examples of this are described. In Figure 13 a subject swallows during scan acquisition and in Figure 14 a subject with chronic obstructive pulmonary disease (COPD) has laboured breathing at rest with marked use of accessory muscles of respiration.

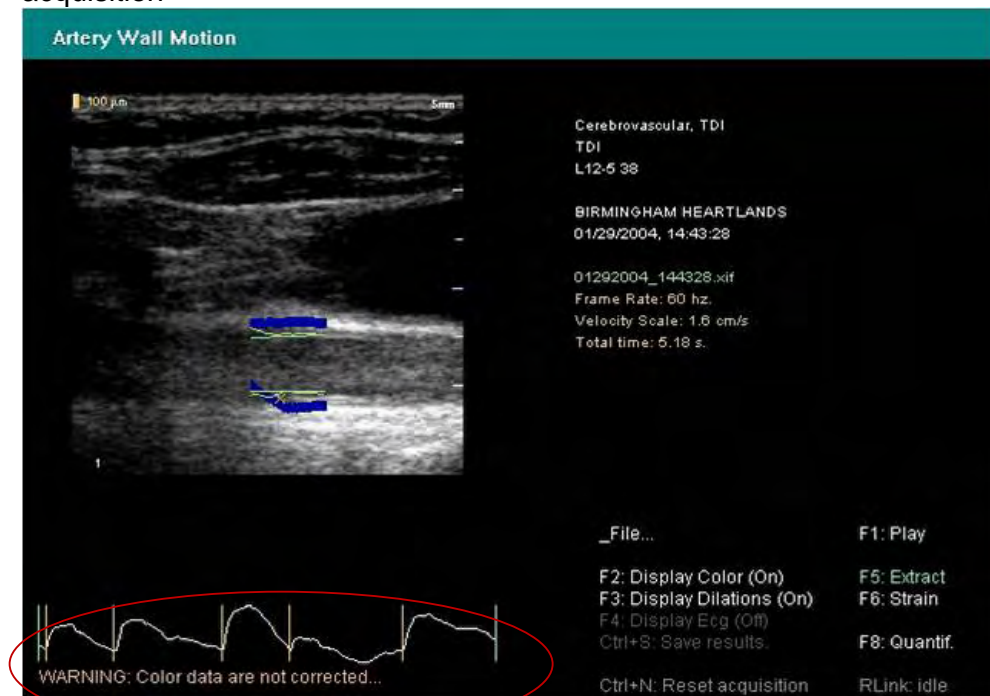


**Figure 13:** Screen capture of a subject swallowing during scan acquisition



**Legend:** subject swallows at end of data capture demonstrating marked change in distension waveform, highlighted within the red circle.

**Figure 14:** Screen capture of a subject breathing heavily during scan acquisition

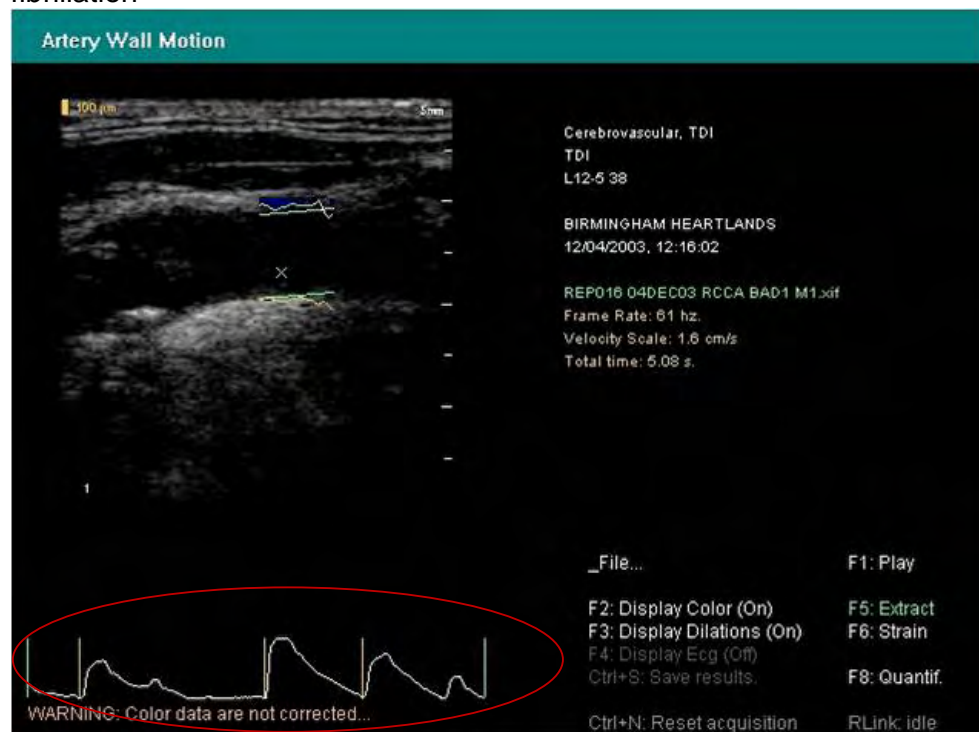


**Legend:** As a result of laboured breathing with use of accessory muscles in this subject, the TDI system is not able to accurately measure the ADW as highlighted in red.

## Cardiac arrhythmias

Due to the variability of beat to beat cardiac output in patients with cardiac arrhythmias such as atrial fibrillation, the pattern and maximum excursion of the ADW was extremely variable and thus unreliable. This is demonstrated in Figure 15. We also found that it was not possible to capture enough cardiac cycles in subjects with bradycardia due to the memory capacity of the ultrasound system, which was big enough only to capture 5 seconds of high resolution images.

**Figure 15:** Screen capture of scan acquisition of a subject with atrial fibrillation



**Legend:** In the presence of an arrhythmia, the TDI system is unable to identify the cardiac cycles, as highlighted in red.

### ***Analysis of BCCOV***

After rejection of the 70 scans with failed wall recognition, subgroup analysis of the remaining scans, sorted by presence of no identified error, arrhythmia present or extra arterial movement present, confirmed that BCCOV was less when these two errors were not identified. These data are presented in Table 5.

**Table 5:** Between cycle coefficient of variation (BCCOV) for 130 scans of the CCA from 65 subjects broken down by error type.

<b>error type</b>	<b>number</b>	<b>BCCOV (%)</b>
<b>no error identified</b>	74	5.62 (3.64 - 7.60)
<b>extra-arterial movement</b>	48	7.23 (1.21 – 15.67)
<b>arrhythmia</b>	8	20.99 (1.69 - 40.29)

---

**Legend:** Figures in brackets are the range calculated as the mean  $\pm$  one SD.

## Conclusions

From this initial study and from information already available about the AWM system, the following criteria were developed to be used in further studies:

- arterial diameter greater than 3 mm
- no side branches
- ensure subject is rested and comfortable on couch prior to scan acquisition for 5 minutes
- exclude subjects with breathing difficulties
- exclude subjects with cardiac arrhythmias
- ensure scan plane alignment in ROI

## A REPRODUCIBILITY STUDY OF A TDI BASED METHOD TO CALCULATE INDICES OF ARTERIAL STIFFNESS

*The aim of the study was to investigate the reproducibility of estimation of Young's modulus  $E$  and pressure strain elastic modulus  $E_p$ , derived from a tissue Doppler imaging (TDI) wall motion technique.*

*Healthy subjects had their arteries insonated at the same sitting by two different observers and at two different sittings by the same observer. From 32 subjects in the reproducibility study, within-scan coefficient of variation (CV) was 4.5%. Intraobserver between-scan CV for  $E$  was 12.7% and for  $E_p$  11.0%. Interobserver CVs were 8.3% and 9.3%, respectively.*

*TDI is a reproducible, valid and highly sensitive direct assessment of arterial wall parameters. It is at least as reproducible as other ultrasound based methods for assessing arterial stiffness and also provides increased information about the arterial distension waveform.*

## Introduction

The aim of this study was to investigate the reproducibility of the TDI system in calculating  $E$  and  $Ep$  in the common carotid arteries (CCAs) of human subjects.

The hypothesis was that given that the TDI system is able to measure AWM in high resolution, it should provide estimations of  $E$  and  $Ep$  that are more reproducible than other available ultrasound based systems for calculating these parameters.

## Methods

### *Study Design*

All subjects recruited into the study provided fully informed written consent. Ethical approval was granted by Birmingham East District Research and Ethics Committee. All subjects completed a questionnaire regarding past medical history, drug evidence and exercise tolerance. A subject was defined as healthy if there was no evidence of cardiovascular disease or associated disease states.

Inter-observer and intra-observer reproducibility was assessed in healthy subjects. For inter-observer reproducibility, the same patient was assessed by two different observers at the same sitting. One observer had 1 years experience in ultrasonography, and the other had 8 years experience. Each observer was unaware of the measurements of the

other observer. For intra-observer reproducibility, the same subject was assessed by the same observer at two sittings on a different day at least 24 hours apart. Each assessment consisted of scan acquisition of the left and right common carotid artery, and blood pressure measurement as described on page 54. A B-mode image of the insonated artery was taken at each scan acquisition to ensure that the same segment of artery was insonated repeatedly. MADC, internal arterial diameter and IMT were assessed separately and  $E$  and  $E_p$  were calculated separately using these data for each CCA.

### ***Statistical analysis***

After extraction and collation of the raw data using Microsoft Excel, the data were analysed using Stata 8.1 for Windows (STATA Stata Corporation, College Station, Texas, USA) in association with a statistician from the University of Birmingham. The results of the reproducibility study were analysed with Bland-Altman Plots and by calculating coefficients of variation. The coefficient of variation was defined as observer error (standard deviation of the mean difference between observations/ $\sqrt{2}$ ) multiplied by 100 divided by the pooled mean values.

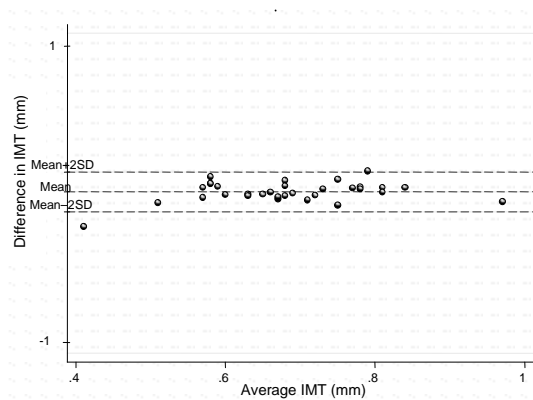
## **Results**

A total of 32 subjects were analysed in the inter-observer study and 30 subjects were analysed in the intra-observer study. The mean age of the

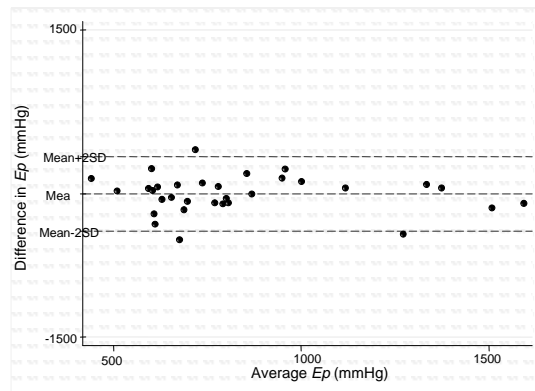
study population was 43 years (range 18 – 72). The mean systolic blood pressure in the study group was 124 mmHg (range 94 – 184) and the mean diastolic blood pressure 75 mmHg (range 54 – 100). The Bland-Altman plots of the inter-observer differences (Figure 16) and intra-observer differences (Figure 17) showed that no relationship exists between the difference in measurement and the estimated true value. The within scan standard deviation of the difference between the measurements was 18 microns for the inter-observer study and 20 microns for the intra-observer study. The within scan (variation between cardiac cycles with one scan) coefficient of variation (standard deviation/mean, CV) was 14.5%. Reproducibility statistics between scans are summarised in Table 6.



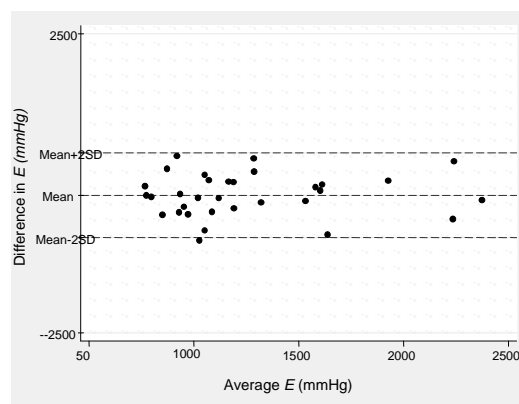
**Figure 16: Inter-observer Bland Altman plots**  
a) Inter-observer plot of IMT variability



b) Inter-observer plot of  $E_p$  variability



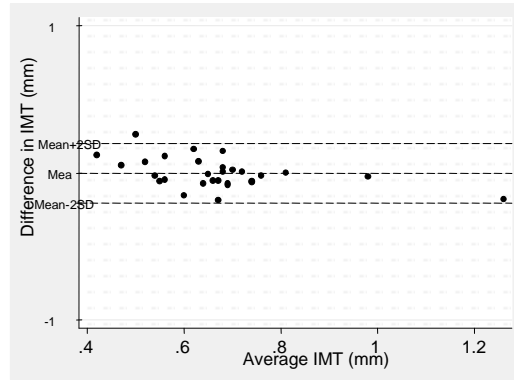
c) Inter-observer plot of  $E$  variability



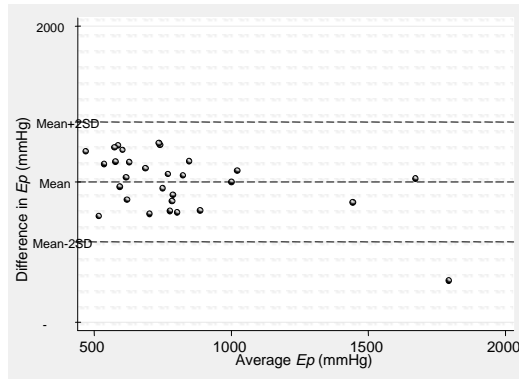
**Legend:** Bland-Altman plots showing inter-observer variability for calculated indices as measured in 32 arteries by two ultrasonographers. The difference between the two measurements plotted on the y-axis against the average of the two measurements on the x-axis. The horizontal lines depict the mean difference (bias) and the limits of agreement (bias  $\pm 2$  SD) between the measurements.

**Figure 17:** Intra-observer Bland Altman plots

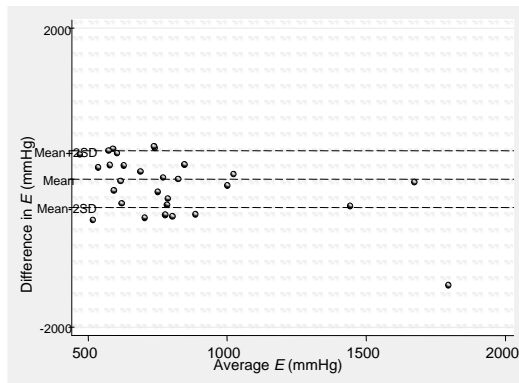
a) Intra observer plot of IMT variability



b) Intra observer plot of  $Ep$  variability



c) Intra observer plot of  $E$  variability



**Legend:** Bland-Altman plots showing intra-observer variability for calculated indices as measured in 32 arteries by two ultrasonographers. The difference between the two measurements plotted on the y-axis against the mean of the two measurements on the x-axis. The horizontal lines depict the mean difference (bias) and the limits of agreement (bias  $\pm 2$  SD) between the measurements.

**Table 6:** Mean values and CV for indices measured in reproducibility study.

	Inter-	observer	study	Intra-	observer	study
	LCCA	RCCA	combined	LCCA	RCCA	combined
<b>d</b> <b>(mm)</b>	6.14* (5.27-8.65) 6.12† (5.08-8.70) <b>2.2%</b> <i>0.19</i>	6.37* (5.19-8.61) 6.37† (5.28-8.32) <b>3.2%</b> <i>0.29</i>	6.25* (5.36-8.42) 6.25† (5.23-8.43) <b>2.1%</b> <i>0.18</i>	6.33‡ (5.08-8.70) 6.20§ (5.19-8.79) <b>3.3%</b> <i>0.30</i>	6.45‡ (5.37-8.61) 6.43§ (5.39-8.42) <b>2.5%</b> <i>0.23</i>	6.38‡ (5.51-8.43) 6.31§ (5.51-8.54) <b>2.1%</b> <i>0.18</i>
<b>IMT</b> <b>(mm)</b>	0.70* (0.51-1.05) 0.70† (0.52-1.16) <b>4.6%</b> <i>0.05</i>	0.66* (0.23-0.94) 0.67† (0.27-0.84) <b>5.8%</b> <i>0.05</i>	0.68* (0.37-0.94) 0.69† (0.46-0.99) <b>4.0%</b> <i>0.03</i>	0.70‡ (0.40-1.63) 0.69§ (0.51-1.16) <b>7.5%</b> <i>0.07</i>	0.63‡ (0.21-1.12) 0.63§ (0.35-1.14) <b>8.3%</b> <i>0.07</i>	0.67‡ (0.39-1.38) 0.66§ (0.44-1.15) <b>6.3%</b> <i>0.07</i>
<b>Ep</b> <b>(mmHg)</b>	116* (63-205) 106† (57-226) <b>12.3%</b> <i>19.4</i>	115* (56-246) 109† (58-301) <b>13.5%</b> <i>21.4</i>	116* (59-198) 107† (58-226) <b>9.3%</b> <i>14.8</i>	119‡ (57-515) 116§ (57-249) <b>12.0%</b> <i>19.9</i>	107‡ (58-301) 102§ (56-236) <b>14.6%</b> <i>21.4</i>	112‡ (58-325) 109§ (444-1651) <b>11.0%</b> <i>34.8</i>
<b>E</b> <b>(mmHg)</b>	516* (237-814) 473† (221-996) <b>13.4%</b> <i>94.2</i>	574* (813-1319) 573† (301-1424) <b>17.3%</b> <i>136</i>	545* (283-980) 523† (261-1059) <b>8.3 %</b> <i>25.7</i>	551‡ (221-1254) 528§ (285-1118) <b>14.9%</b> <i>114</i>	571‡ (273-1384) 531§ (285-1068) <b>17.3%</b> <i>134</i>	561‡ (287-1039) 530§ (301-1093) <b>12.7%</b> <i>32.6</i>

**Legend:** \* first observer; †second observer; ‡ first session; § second session; value in bold = coefficient of variation; values in brackets = range; value in italics = standard deviation; IMT = intima-media thickness; Ep = Elastic modulus; E = Young's modulus.

## Discussion

This study has demonstrated for the first time that TDI is a reproducible and valid method of analysing the elastic properties of arteries. The reproducibility of our measurements is at least as good as the best reported in the literature using conventional B-mode based techniques<sup>240</sup>. The reproducibility is also improved when compared to methods using echo tracking.<sup>241</sup> Ultrasound is often criticised for its operator

dependence. This study demonstrated excellent reproducibility between both observers, one of whom had only one year's experience. TDI scans along many points of the segment of artery of interest at a higher frame rate than other ultrasound based methods, and we believe this is the main reason behind the improved results. Central pulse wave velocity estimation demonstrates similar reproducibility to our method, but is not a direct assessment of arterial stiffness.<sup>242</sup> The range of values for  $E$  and  $E_p$  that we have calculated in healthy subjects is similar to that estimated in previous studies.<sup>243</sup>

We have shown that the left CCA demonstrated superior reproducibility to the right. The left CCA is anatomically the most closely related to the aorta, arising directly from the aortic arch, as opposed to the right CCA which arises indirectly from the aorta via the brachiocephalic trunk. Thus the left CCA is thought to be most likely to reflect the qualities of the central aorta. We believe that the left CCA is the best site to measure CCA stiffness, particularly when the results are used as a measure of systemic arterial stiffness.

## Conclusions

TDI is a reproducible, valid and highly sensitive direct assessment of arterial wall stiffness, which suggests that the increased stiffness in subjects with PAD is not just a function of increased wall thickness or diameter, but possibly a change in arterial wall structure as well.

## RELATIONSHIP BETWEEN AGE AND ARTERIAL STIFFNESS

*It remains difficult to decide whether the cardiovascular effects of increased age are separate to that of atherosclerosis. Increased arterial stiffness, PP and IMT have all been shown to be associated with increased age. The aim of this chapter was to examine the relationship between E, Ep, PP, IMT and increased age in a cohort of healthy subjects.*

*The arterial distension waveform, IMT, diameter and brachial blood pressure were measured to calculate E, Ep in the common carotid arteries of subjects. 121 subjects (age 5-83 years) were examined. E, Ep, SBP, DBP, d (all  $p < 0.0001$ ), IMT ( $p = 0.014$ ) and PP ( $p = 0.002$ ) were all significantly associated with increased age. The regression coefficient was greater for E (10.64) and Ep (2.59) than for PP (0.161), IMT (0.001), SBP (0.45), DBP (0.355) and d (0.023). Correlation with age was higher for E (0.71) and Ep (0.81) than PP (0.19), IMT (0.24), SBP (0.59), DBP (0.59) and d (0.52). This change was maintained across all percentiles for all parameters.*

*We have shown for the first time in a cross sectional study that E and Ep have a stronger relationship with increased age than IMT or PP, early markers of atherosclerosis. This suggests that the first changes in ageing may be the development of increased arterial stiffness rather than atherosclerosis.*

## Introduction

Qualitative changes in cardiovascular physiology with age have been recorded for over one hundred years.<sup>15,75</sup> Interest in the quantitative limits of blood pressure gained favour over the qualitative form with the emergence of the cuff sphygmomanometer. It is only more recently that interest in the qualitative form of arterial blood pressure has once again emerged with the study of arterial stiffness.

There is controversy surrounding much recent research concerning vascular ageing. The reasons for this include a lack of an accurate animal model to simulate human cardiovascular ageing,<sup>76-79</sup> inaccuracy of non-invasive investigations, difficulty in discriminating the difference between natural ageing and occult disease, controversy over what the natural ageing process actually is, and difficulty in identification of the reserve physiological capability of cardiovascular and other organs. However, the main functional change with increasing age appears to be progressive stiffening of the central aorta with subsequent effect on cardiac function.<sup>15,83</sup> Increased aortic stiffness with age has been found in both western populations with a high propensity towards atherosclerosis and other populations not prone to atherosclerosis.<sup>84-88</sup> It is therefore believed to be a genuine age dependent change. At any particular age there is a wide range of arterial stiffness.<sup>89</sup>

Increased carotid IMT has been shown to be a good indicator of generalised atherosclerosis,<sup>54</sup> as well as a predictor of cardiovascular

events.<sup>244-246</sup> This relationship is presumed to exist as increased carotid IMT represents mainly increased intimal thickening in the elastic carotid artery.<sup>54</sup> Similarly increased CCA diameter has been shown to be a marker of atherosclerotic change.<sup>247</sup> However, increased IMT and diameter may be late markers of atherosclerotic changes in the arterial wall.

PP is the difference between systolic and diastolic blood pressure. It has long been recognized as a valuable surrogate marker of arterial stiffness<sup>15</sup> as it depends on cardiac output, large artery stiffness and wave reflection. Both SBP and DBP increase with age. However, beyond the sixth decade, DBP does not increase whereas SBP continues to do so. Thus pulse pressure increases with age.<sup>13</sup> PP takes no account of change in volume and therefore is not a true measure of arterial stiffness. Data from the Framingham study demonstrates that PP predicts the risk of coronary heart disease better in a population over the age of 50 years than SBP or DBP measurements alone.<sup>18</sup>

The aim of this study was to investigate indices of arterial stiffness, blood pressure and IMT in the CCAs of healthy subjects of different ages.

The hypothesis was that indices of arterial wall stiffness would increase more than IMT with age in healthy subjects.

## **Methods**

### ***Study design***

All subjects recruited into the study provided fully informed written consent. Ethical approval was granted by Birmingham East District Research and Ethics Committee. All potential subjects were screened using a questionnaire regarding past medical history and drug evidence.

The relationship between age and arterial stiffness was measured in healthy volunteers. Subjects recruited were either members of staff at Heart of England NHS Trust or were known to them. All subjects answered a detailed questionnaire to look for evidence of disease and all potential subjects with any significant past medical history were excluded from the study. Therefore, only subjects who had no history of diabetes, hypertension, cardiovascular disease (including PAD, CAD and CVD), chronic kidney disease (CKD) and respiratory disease were included in the study. In addition subjects were not accepted into the study if they were taking any form of regular medication.

### ***Statistical analysis***

After extraction and collation of the raw data using Microsoft Excel, the data were analysed using Stata 8.1 for Windows (STATA Stata Corporation, College Station, Texas, USA).



## Results

120 subjects were recruited ranging from 5 to 83 years of age. 50 (43%) were male. Mean SBP was 123 mmHg (range 89-169 mmHg) and mean DBP was 73mmHg (range 41-110mmHg). These demographics are further detailed in Table 7 and segregated per decade, together with mean values per decade for PP,  $d$ , IMT,  $E$  and  $Ep$ . We found that  $E$ ,  $Ep$ , SBP, DBP,  $d$  (all  $p < 0.0001$ ), IMT ( $p = 0.014$ ) and PP ( $p = 0.002$ ) were all significantly associated with increasing age when analysed with linear regression. The regression coefficient was greater for  $E$  (10.64) and  $Ep$  (2.59) than for PP (0.161), IMT (0.001), SBP (0.45), DBP (0.355) and  $d$  (0.023). In addition the correlation with age was also higher for  $E$  (0.71) and  $Ep$  (0.81) than PP (0.19), IMT (0.24), SBP (0.59), DBP (0.59) and  $d$  (0.52). These data are presented in Table 8. Regression plots of these relationships are found in Figures 18-24.

**Table 7: Main characteristics of study population split into decades**

age (years)	no	male	SBP (mmHg)	DBP (mmHg)	PP (mmHg)	d (mm)	IMT (mm)	E (kPa)	Ep (kPa)
0-9	21	7 (33)	108 (88-125)	60 (42-96)	47 (23-62)	5.6 (4.6-6.6)	0.67 (0.58-0.93)	207 (73-536)	49 (23-119)
10-19	40	23 (57)	119 (103-143)	63 (50-80)	55 (37-70)	5.7 (4.5-7.5)	0.69 (0.45-1.03)	282 (106-533)	64 (41-131)
20-29	12	4 (33)	117 (99-133)	68 (58-85)	49 (35-66)	6.6 (5.1-7.8)	0.72 (0.59-1.33)	377 (229-556)	79 (49-97)
30-39	24	7 (29)	121 (104-143)	69 (57-94)	51 (33-63)	6.4 (5.1-7.2)	0.70 (0.56-0.96)	477 (269-1296)	101 (64-200)
40-49	11	4 (36)	127 (120-137)	74 (65-83)	53 (38-69)	6.7 (5.9-8.2)	0.68 (0.48-0.94)	660 (231-1303)	123 (71-193)
50-59	5	3 (60)	131 (111-146)	79 (64-96)	53 (48-57)	7.1 (6.4-8.3)	0.73 (0.62-0.83)	757 (364-1299)	153 (89-265)
60-69	3	1(33)	144 (114-170)	81 (65-90)	63 (49-80)	6.4 (5.8-7.0)	0.88 (0.68-1.05)	682 (542-813)	188 (127-229)
70-79	2	1 (50)	141 (119-162)	86 (75-86)	60 (44-77)	6.7 (6.5-6.9)	0.84 (0.76-0.93)	1045 (731-1359)	253 (207-298)
80-89	2	1 (50)	170 (163-177)	85 (73-95)	84 (73-95)	6.5 (5.8-7.3)	0.91 (0.91-0.93)	785 (785-890)	317 (245-390)

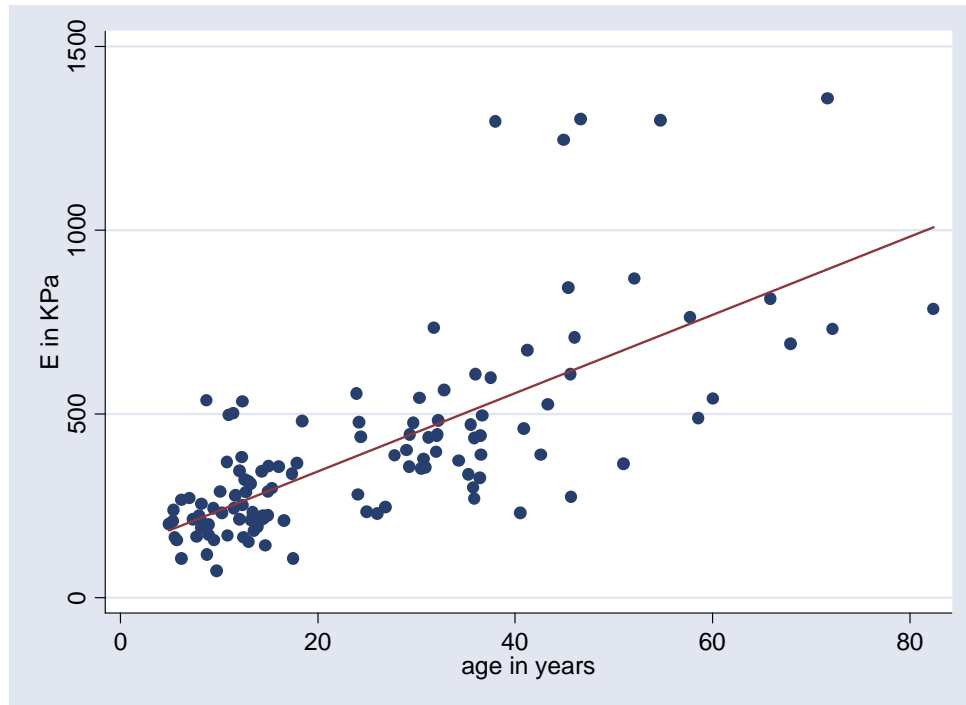
**Legend:** figures are mean values per decade with 95% confidence intervals in brackets, except for male category where figure in brackets is percentage per decade; SBP, DBP and PP in mmHg; d and IMT in mm; E and Ep in KPa.

**Table 8: Regression coefficients and correlation between experimental variables and age**

variable	regression coefficient	t	p value	R <sup>2</sup>	correlation with age
E	10.64	11.02	<0.0001	0.51	0.71
Ep	2.597	15.48	<0.0001	0.67	0.81
PP	0.161	3.14	0.002	0.07	0.19
SBP	0.45	7.45	<0.0001	0.37	0.59
DBP	0.355	7.46	<0.0001	0.36	0.59
IMT	0.001	2.50	0.014	0.04	0.24
diameter	0.023	6.69	<0.0001	0.27	0.52

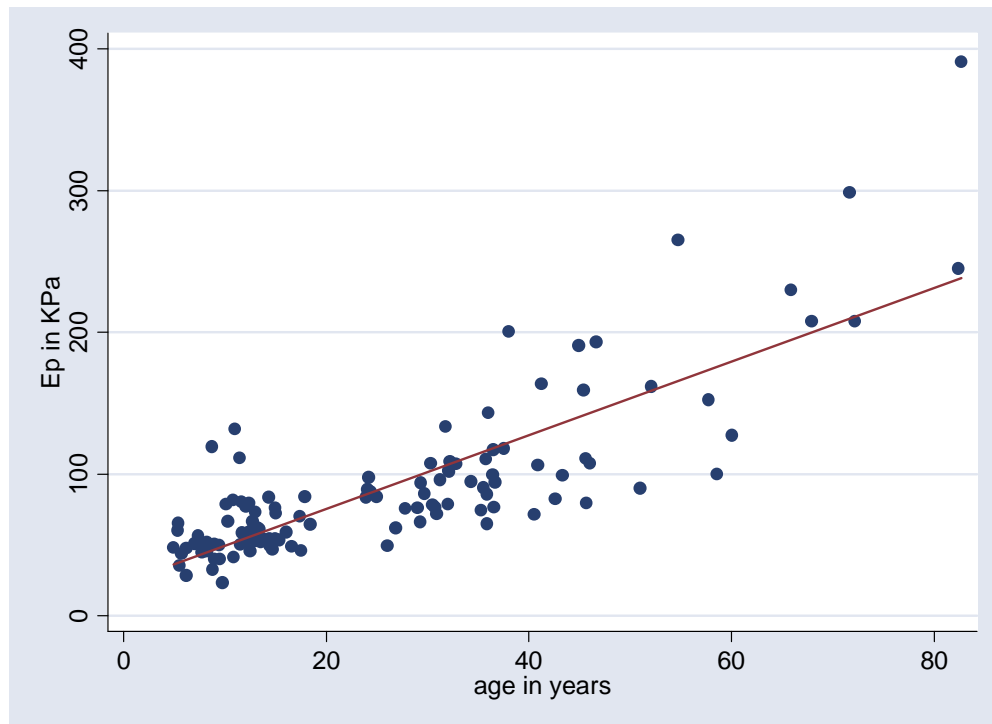
**Legend:** PP=pulse pressure (mmHg); IMT=intima-media thickness (mm); E= Young's modulus (KPa); Ep=elastic modulus (KPa); SBP=systolic blood pressure (mmHg); DBP=diastolic blood pressure (mmHg); d=diameter (mm).

**Figure 18:** Regression graph of Young's modulus plotted against age



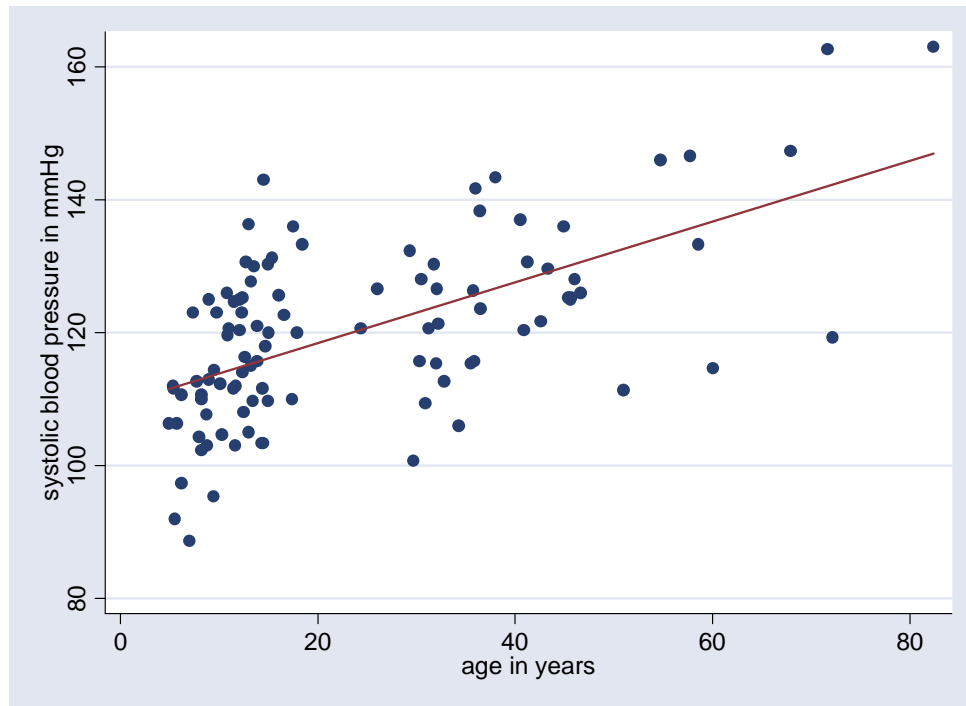
**Legend:** red line is plot of linear regression of Young's modulus against age

**Figure 19:** Regression graph of Peterson's elastic modulus plotted against age



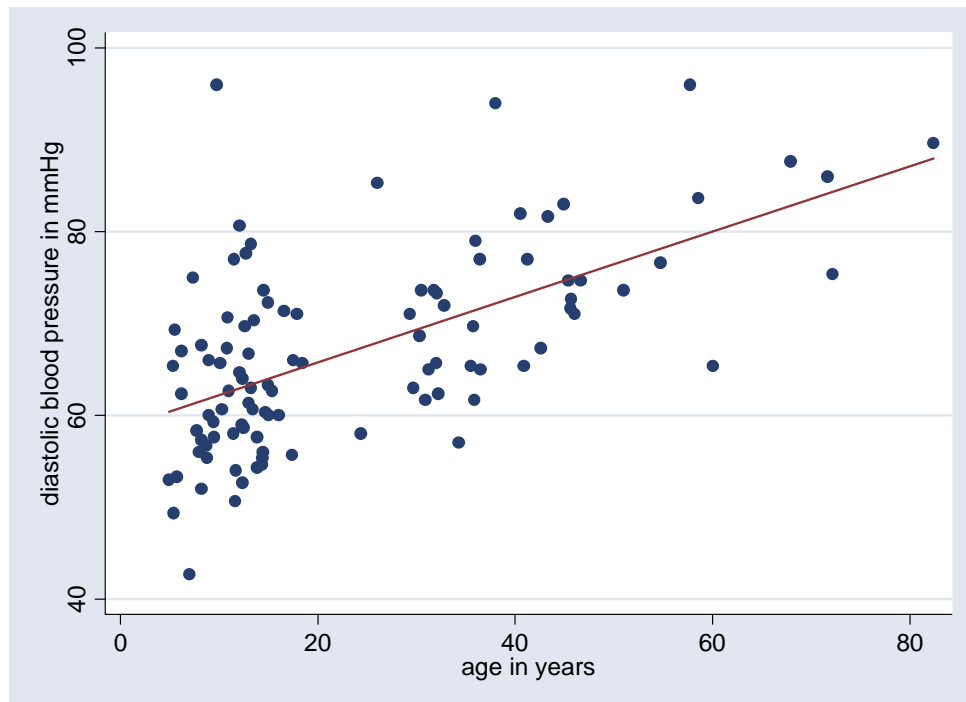
**Legend:** red line is plot of linear regression of Peterson's elastic modulus against age

**Figure 20:** Regression graph of systolic blood pressure plotted against age



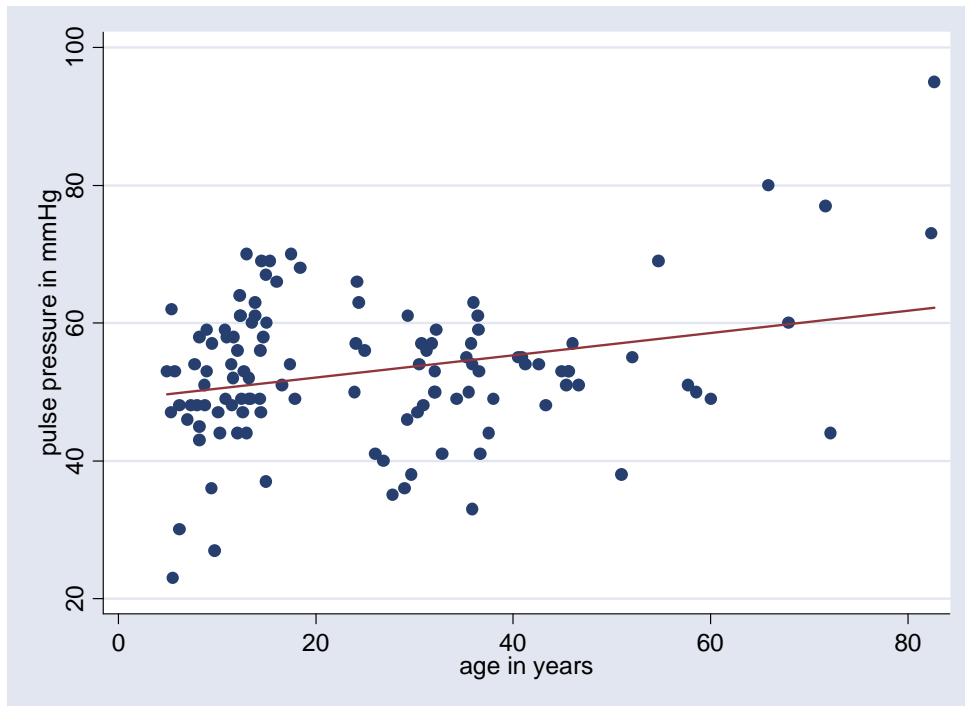
**Legend:** red line is plot of linear regression of systolic blood pressure against age

**Figure 21:** Regression graph of diastolic blood pressure plotted against age



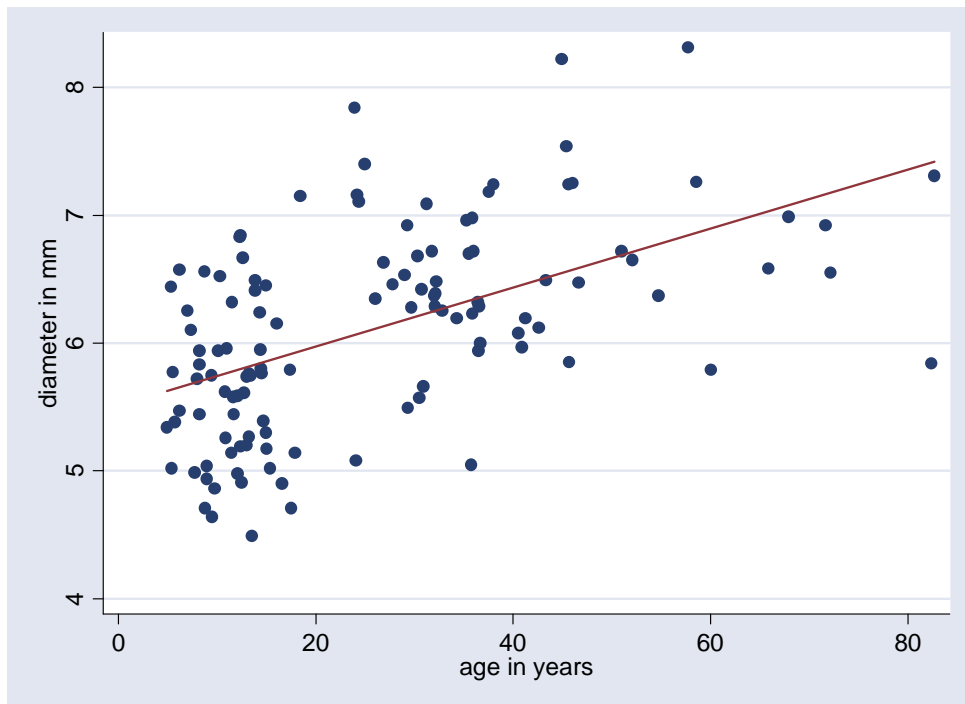
**Legend:** red line is plot of linear regression of diastolic blood pressure against age

**Figure 22:** Regression graph of pulse pressure plotted against age



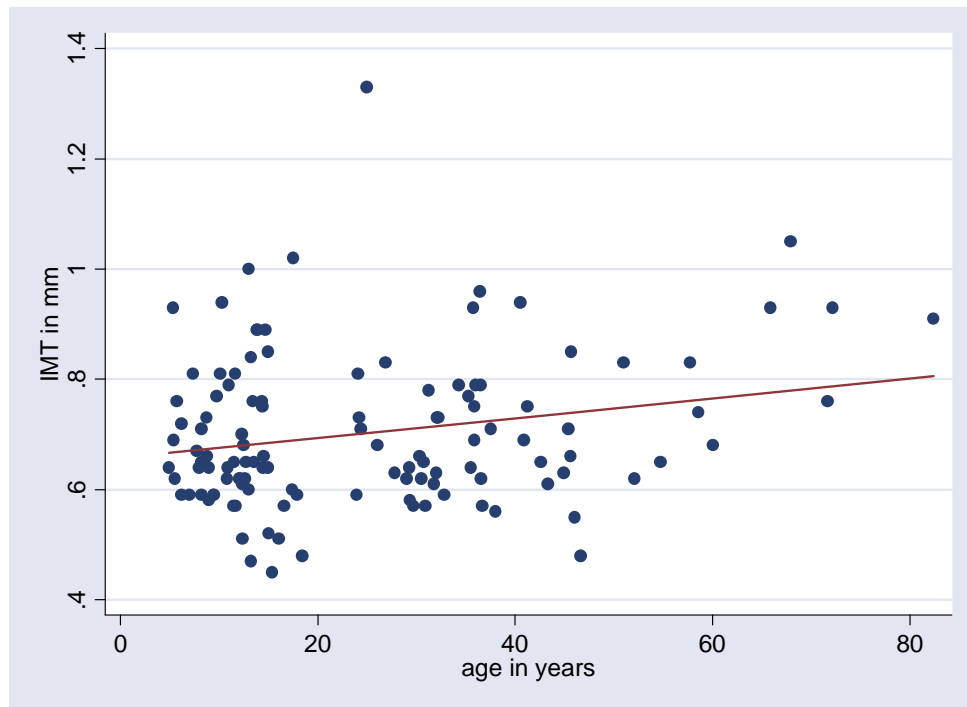
**Legend:** red line is plot of linear regression of pulse pressure against age

**Figure 23:** Regression graph of diameter plotted against age



**Legend:** red line is plot of linear regression of diameter against age

**Figure 24:** Regression graph of IMT plotted against age



**Legend:** red line is plot of linear regression of intima media thickness against age

## Discussion

We have shown that  $E$  and  $Ep$  have the strongest relationship with increased age when compared to early markers of atherosclerosis (IMT and PP) in a cross sectional study of healthy subjects with an age range of 5 to 83 years. We believe that this suggests that the first cardiovascular changes in ageing may be increased arterial stiffness rather than these changes being secondary to the development of atherosclerosis. These findings would appear to fit with the histological observations that the most significant arterial change with ageing is in the arterial media, where there is loss of the orderly arrangement of elastin fibres which also undergo thinning splitting, fraying and fragmentation together with an increase in collagen and ground substance,<sup>82</sup> and a decrease in the amount of smooth muscle.<sup>82</sup> Atherosclerosis is predominantly a disease of the intima and we would have expected to observe a stronger relationship between IMT and age if the major change in cardiovascular ageing was atherosclerosis. We accept that the only place that we have measured IMT is in the CCA. However this is the most commonly measured site, and is a well recognised marker for early atherosclerotic change.<sup>239</sup>

In line with other studies of this nature we have used data from the left CCA only. This artery demonstrated superior reproducibility to the right in our reproducibility study.<sup>248</sup> The left CCA is anatomically the most closely related to the aorta arising directly from the aortic arch as

opposed to the right CCA which arises indirectly from the aorta via the brachiocephalic trunk. Thus the left CCA is thought to be most likely to reflect the qualities of the central aorta. In addition, previous research only supports evidence of increased arterial stiffness in the aorta and other central elastic arteries, with little evidence being found for a change in stiffness with age in the muscular arteries such as the brachial, radial or femoral,<sup>36,86,90</sup> so we believe that the left CCA is a good site to measure arterial stiffness in this particular study.

Our aim was only to recruit healthy volunteers into the study. This is difficult to achieve without detailed testing of each potential recruit. We believe that we achieved this aim via stringent screening criteria, but we accept that subjects with occult disease may still have been accepted into the study. Serum lipid profiles could help in identifying potential subjects with occult cardiovascular disease, but these were not taken in this study population. We recognise that this cross sectional study may only include survivors in any age group, particularly with increasing age who are able to withstand the effects of cardiovascular ageing.<sup>80</sup> However a prospective cohort study would be the only way to confirm or refute this, which would necessarily take at least a lifetime to complete.

The DBP rose with age in our study population, which was not expected, as most studies examining blood pressure and age have found that it decreases with age. This may be a sampling error.



## Conclusions

We have shown that markers of arterial stiffness are more increased with increased age than markers of atherosclerosis. We believe that this study provides further evidence that the process of cardiovascular ageing is associated with increased arterial stiffness and subsequent functional change as a result of a 'worn out' arterial media. It is possible that this process is separate from atherosclerosis, although may predispose an individual to develop it.

## MEASUREMENT OF ARTERIAL STIFFNESS IN SUBJECTS WITH VASCULAR DISEASE: ARE VESSEL WALL CHANGES MORE SENSITIVE THAN INCREASE IN INTIMA-MEDIA THICKNESS?

*It is widely accepted that subjects with vascular disease have increased arterial stiffness and IMT when compared with healthy controls. The aim of this study was to investigate indices of arterial stiffness and IMT in the CCAs of subjects with and without PAD, in order to look for evidence of change in wall quality and quantity to explain increased stiffness that has been found in the arteries of subjects with vascular disease.*

*The ADW, IMT, d and BP were measured to calculate E and Ep in 38 subjects with confirmed PAD and 43 normal controls matched for age, sex, smoking and hypertension. The mean diameter (8.35mm [95% CI 7.93–8.77] vs. 6.93mm [6.65–7.20]  $P < 0.001$ , increase 20%), IMT (0.99mm [0.92–1.07] vs. 0.88mm [0.82–0.93]  $P = 0.020$ , increase 12.5%), Ep (315 kPa [185–444] vs. 190 kPa [164–216]  $P = 0.034$ , increase 66%) and E (1383 kPa [836–1930] vs. 744 kPa [641–846]  $P = 0.006$ , increase 86%) were all significantly higher in subjects with PAD.*

*This study suggests that increased stiffness observed in subjects with peripheral vascular disease is a result of change in both quantity and quality of the arterial wall. Changes in indices of arterial stiffness were much higher than changes in IMT and diameter. These preliminary observations may be an indication that indices of arterial stiffness are a sensitive early marker of atherosclerosis.*

## Introduction

An enlarging body of evidence suggests that increased arterial stiffness is associated with markers of cardiovascular risk and that this stiffness may herald the onset of cardiovascular disease before manifestation of symptoms or detection of frank atherosclerotic lesions.<sup>249,250</sup> Measurement of arterial stiffness could become a part of the process of both risk assessment and monitoring of therapy in patients with cardiovascular disease.

Patients with atherosclerosis have changes in the arterial wall, such as the formation of atherosclerotic plaques together with a varying degree of in situ thrombosis, and fibrosis and calcification of the tunica media. Increased wall thickness is also often demonstrated.<sup>239</sup> PAD is usually a reflection of a systemic atherosclerotic disease.<sup>251</sup> We hypothesise that material stiffness changes at a different rate from structural stiffness in patients with and without PAD.

The aim of this study was to investigate markers of material and structural arterial stiffness ( $E$  and  $Ep$ ) and wall thickness (IMT) in the CCAs of human subjects with and without evidence of PAD in order to study which variable changes the most in affected subjects.

The hypothesis was that changes in stiffness parameters of the arterial wall would be greater than changes in IMT in subjects with PAD when compared to a healthy matched control population.

## **Methods**

### ***Study design***

All subjects recruited into the study provided fully informed written consent. Ethical approval was granted by Birmingham East District Research and Ethics Committee. All subjects completed a questionnaire regarding past medical history and drug evidence.

### ***Subject Selection***

The elastic properties of CCAs in age- and sex- matched subjects with and without evidence of PAD were compared. Subjects with PAD were recruited from a dedicated nurse led outpatient clinic at the Heart of England NHS Foundation Trust for subjects with non disabling lower limb claudication, who had opted to have conservative treatment for their symptoms. Their claudication symptoms were stable for at least 6 months. Prospective control population subjects were recruited from non vascular outpatient clinics at the same hospital. These subjects were attending hospital with non cardiovascular problems such as herniae, simple skin lesions and benign urological disease. All participants were subject to similar scrutiny in order to determine their vascular status. Subjects were deemed free from PAD if they had an unlimited walking distance and an ABPI greater than 0.9. Similarly all patients with PAD

had a confirmed history of lower limb claudication and an ABPI of less than 0.9.

### ***Statistical analysis***

After extraction and collation of the raw data using Microsoft Excel, the data were analysed using Stata 8.1 for Windows (STATA Stata Corporation, College Station, Texas, USA) in association with a statistician from the University of Birmingham. Mean values in the case population and the control population were compared using Student's *t*-test after log transformation.

## Results

We assessed the left common carotid artery (LCCA) in 38 cases with evidence of PAD and 43 healthy controls. Both populations were well matched for age, sex and co-existing risk factors as demonstrated in Table 9. Co existing medication is listed in Table 10. As can be seen, the PAD group had a higher incidence of Statin use than the control group, but otherwise there is no other significant difference in cardiovascular medication between the two groups. Cases with PAD had a significantly increased  $E$  and  $Ep$ , diameter and IMT compared to subjects without PAD (Table 11). Interestingly, the increases in indices of stiffness, in particular material stiffness seen in subjects with PAD were higher compared to the increases seen in IMT and diameter.  $E$  was found to be 86% greater in the PAD group compared to the control group and  $Ep$  66% greater. In comparison, diameter was only 20% greater and IMT 12.5% greater. All the results were statistically significant when analysed using Students t-test after log transformation.

**Table 9:** Main characteristics of study populations.

	No.	Age (range)	Male (%)	SBP (95% CI)	Smoking (%)	↑BP (%)	CAD (%)	CVD (%)	DM (%)
<b>PAD</b>	38	69 (55-85)	32 (84)	154 (106-203)	25 (68)	20 (52)	3 (8)	2 (5)	6 (16)
<b>Control</b>	43	67 (55-82)	33 (77)	154 (110-203)	30 (69)	17 (40)	4 (9)	3 (7)	5 (12)

**Legend:** PAD= peripheral arterial disease; SBP = systolic blood pressure; ↑BP= hypertension; CAD=coronary artery disease; CVD= cerebrovascular disease; DM= diabetes mellitus

**Table 10:** concurrent drug therapy for study populations

	No. of subjects	Statin	diuretic	ca antagonist	nitrate	B-blocker	ACE-I
<b>PAD</b>	38	28 (74)	8 (21)	6 (16)	2 (5)	2 (5)	0 (0)
<b>Control</b>	43	6 (14)	10 (23)	6 (14)	4 (9)	5 (12)	6 (14)

**Legend:** ACE-I = angiotensin converting enzyme inhibitor; figure in brackets is percentage

**Table 11:** Wall properties of study populations.

	no. of subjects	IMT mm	diameter mm	Ep KPa	E KPa
<b>PAD</b>	38	0.99 (0.92-1.07)	8.35 (7.93-8.77)	315 (185 – 444)	1383 (836-1930)
<b>control</b>	43	0.88 (0.82-0.93)	6.93 (6.65-7.20)	190 (164 – 216)	744 (641 – 846)
<b>increase</b>		12.5%	20%	66%	86%
<b>p value</b>		0.020	< 0.001	0.034	0.006

**Legend:** IMT = intima-media thickness; Ep = Elastic modulus; E= Young's modulus; mean values and 95% confidence intervals in brackets; p values calculated with the Student's t-test after log transformation

## Discussion

We have shown that arterial diameter, IMT,  $E$  and  $Ep$  are significantly increased in subjects with PAD compared to controls without PAD. We are not aware of any study that has compared  $E$  and  $Ep$  in subjects with and without PAD. Differences in arterial stiffness between subjects with and without PAD have been shown before.<sup>150,151</sup> Increased carotid IMT has been shown to be a good indicator of generalised atherosclerosis<sup>54</sup>, as well as a predictor of cardiovascular events.<sup>244-246</sup> This relationship is presumed to exist as increased carotid IMT represents mainly increased intimal thickening in the elastic carotid artery.<sup>54</sup> Similarly increased CCA diameter has been shown to be a marker of atherosclerotic change.<sup>247</sup> However, increased IMT and diameter may be late markers of atherosclerotic changes in the arterial wall. We demonstrated changes in the elastic properties of the arterial wall which are independent variables,<sup>24</sup> and are thought to reflect material as well as structural change. The increase in  $E$  in the PAD group was mostly due to the increase in  $Ep$  as the increase in IMT and diameter in that group, as can be seen from the formula for  $E$  (page 12), partly balance each other out.

Our findings suggest that both  $E$  and  $Ep$  increase more than IMT and diameter in subjects with PAD. The change in  $E$  (86%) was nearly seven times greater than IMT (12.5%) and more than four times greater than diameter (20%) when compared to controls. Similarly, the change in  $Ep$  (66%) was more than five times greater than IMT (12.5%) and more



than three times greater than diameter (20%). Based on this, we believe that markers of arterial stiffness, particularly those indicating material change in the arterial wall (such as  $E$ ) could be a more sensitive index of developing atherosclerosis than structural measurements alone such as change in IMT and diameter, that may even change before IMT does. This is because these indices describe the relationship between stress and strain whereas IMT and diameter report the result of a change in this relationship. There is some evidence in the literature to support this hypothesis. Giannattasio et al found that increased arterial stiffness, measured as reduced carotid artery distensibility, was present in normotensive normoglycaemic offspring of type 2 diabetic parents, when compared to a control group.<sup>252</sup> However, there was no change in IMT between the two groups.

As demonstrated in Table 9, the PAD group had a higher incidence of Statin use than the control group as a result of best medical therapy intended to limit progression of known cardiovascular disease and prevent secondary cardiovascular events. We do not believe that this discrepancy affects the result, as published literature suggests that Statin use decreases rather than increases arterial stiffness.<sup>207</sup>

## Conclusions

This study has shown a significant difference in  $E$  and  $Ep$ , diameter and IMT in the CCAs of subjects with and without PAD, which suggests that the increased stiffness in subjects with PAD is not just a function of increased wall thickness or diameter, but a change in arterial wall structure as well. The change in indices of arterial stiffness was much greater than the changes in IMT or diameter. We believe that this is because changes in arterial stiffness are an indication of a change in the relationship between strain and stress on the arterial wall, rather than changes in diameter or IMT which are a result of this change. It is possible therefore that atherosclerotic change may be heralded at an earlier point by noting a change in arterial stiffness rather than a change in the structure of the arterial wall, and we recommend that prospective research is carried out in this area to confirm or refute this.

## ARTERIAL STIFFNESS IN WITH RENAL DISEASE: ARE CHANGES IN THE VESSEL WALL EARLIER MARKERS OF CARDIOVASCULAR DISEASE THAN IMT AND PULSE PRESSURE?

*There is increased cardiovascular mortality in subjects with ESRD. Arterial stiffness in these subjects is increased when compared to a healthy population and has been shown to be a strong predictor of mortality. The aim of this study was to investigate the relationship between markers of cardiovascular disease and subjects with no, mild and severe CKD.*

*The arterial distension waveform, IMT, diameter and brachial blood pressure were measured to calculate  $E$  and  $E_p$  in the common carotid arteries of subjects with and without CKD. Data was available for 15 patients with normal kidney function and no evidence of cardiovascular disease, 29 patients with mild CKD (eGFR 89 to 60) and 24 patients with severe CKD (eGFR < 30). Age, cardiovascular disease, hypertension, hyperlipidaemia, PP,  $E_p$  and  $E$  were all significantly associated with renal disease. Only age,  $E$  and  $E_p$  were significantly different between healthy subjects and those with mild renal impairment. Logistic regression demonstrated that only  $E_p$  and  $E$  were independently associated with mild renal disease compared to normal, in a model adjusting for sex, age and diabetes. Only  $E$  was independently associated with severe renal disease compared to non-severe renal disease in the same model adjusting for age sex and diabetes.*

*$E$  and  $E_p$  may be early markers of cardiovascular disease in subjects with CKD that may manifest change before other more recognized markers such as IMT and PP.*

## Introduction

It is well recognized that subjects with ESRD have increased mortality when compared to healthy controls. With unrestricted access to renal dialysis, this annual mortality of approximately 10% is largely secondary to cardiovascular complications of ESRD rather than death from renal failure per se.<sup>253-255</sup> Subjects with ESRD have been shown to have increased arterial stiffness.<sup>256</sup> In addition increased arterial stiffness has been shown to be a strong independent predictor of both cardiovascular and all cause mortality in these subjects.<sup>180,257,258</sup> It is probable that cardiovascular disease starts to develop early in CKD. Even mild to moderate renal insufficiency is associated with increased risk of developing cardiovascular disease and death from it.<sup>259</sup> Increased arterial stiffness in renal transplant patients appears to correlate strongly with the length of time on dialysis, suggesting that the duration rather than the degree CKD may be more important in the development of cardiovascular disease.<sup>183</sup>

Increased carotid IMT has been shown to be a good indicator of generalised atherosclerosis,<sup>54</sup> as well as a predictor of cardiovascular events.<sup>244-246</sup> This relationship is presumed to exist as increased carotid IMT represents mainly increased intimal thickening in the elastic carotid artery.<sup>54</sup> Similarly increased CCA diameter has been shown to be a marker of atherosclerotic change.<sup>247</sup> However, increased IMT and

diameter may be late markers of atherosclerotic changes in the arterial wall.

The aim of this study was to investigate the relationship between IMT, PP and indices of arterial stiffness in subjects with different stages of CKD in order to gain information as to which markers of cardiovascular disease may change first in patients with renal disease.

The hypothesis was that indices of arterial wall stiffness would increase more than IMT and PP in subjects with increasing severity of CKD.

## **Methods**

### ***Study design***

All subjects recruited into the study provided fully informed written consent. Ethical approval was granted by Birmingham East District Research and Ethics Committee. All subjects completed a questionnaire regarding past medical history and drug evidence.

### ***Subject Selection***

The elastic properties of CCAs in subjects with different stages of CKD were compared. Subjects with CKD were recruited from renal outpatient clinics at the Heart of England NHS Foundation Trust. Prospective healthy subjects were recruited from non renal outpatient clinics at the same hospital. These subjects were attending hospital with non cardiovascular problems such as herniae, simple skin lesions and benign urological disease. All participants were subject to similar scrutiny in order to determine their renal status. No subjects were receiving renal replacement therapy.

### ***Calculation of CKD stage***

The absence of kidney disease was defined by proteinuria of less than 300 mg/day and by a glomerular filtration rate (GFR) above 90 ml/min, demonstrated on samples taken within 6 months of participation in this study. If the GFR was 60-89 mlmin<sup>-1</sup>, kidney function was assumed to be normal if diabetes and hypertension were also absent and a kidney ultrasound demonstrated normal appearances. All other instances suggested the presence of kidney disease. Stages of chronic kidney disease were defined according to the level of GFR as recommended by the National Kidney Foundation guideline Kidney Disease Outcomes Quality Initiative clinical practice guidelines for chronic kidney disease.<sup>260</sup>

The GFR was estimated from a blood sample (serum creatinine), using the Modification of Diet in Renal Disease Study equation,<sup>261</sup> and proteinuria was estimated from a spot urine sample (protein-creatinine ratio).

Subjects were recruited and divided into three distinct groups. Normal (no evidence of kidney or cardiovascular disease), Mild (eGFR 89 to 60 ml/min<sup>-1</sup>, CKD stage 2) and Severe (eGFR less than 30 ml/min<sup>-1</sup>, CKD stage 4 or 5).

### ***Statistical analysis***

After extraction and collation of the raw data using Microsoft Excel, the data were analysed using Stata 8.1 for Windows (STATA Stata Corporation, College Station, Texas, USA).

## **Results**

We assessed elastic properties of the left CCA in 68 subjects, 15 in the normal group, 29 in the mild group and 24 in the severe group. Subject demographics and coexisting morbidity are detailed in Table 12. Univariate analysis demonstrated significant correlation between renal disease and age, cardiovascular disease, hypertension, hyperlipidaemia, PP,  $E_p$  and  $E$ . These relationships were maintained when subjects from the mild and moderate renal failure group were compared, but were only present for age,  $E_p$  and  $E$  when subjects from the normal renal function group and mild renal failure group were compared. IMT was not found to be significantly correlated with renal status in any comparison. These data are presented in more detail in Table 12.

We used logistic regression to look for predictors of change between the Normal group and the Mild group, and the Mild group and the Severe group. The model used in both instances included age, sex and diabetes. We found that  $E$ ,  $E_p$  and age were independently associated when comparing the Normal and Mild group. Notably, IMT and PP were not. Comparing the Mild and Severe group, age and  $E$  were significantly associated, but  $E_p$ , IMT and PP were not. These data are



presented in more detail in Table 13. Box plots of  $E$ ,  $Ep$ , PP and IMT against renal group are shown in Figures 25-28.

**Table 12:** Main characteristics of study population divided per renal group.

characteristic	normal	mild	severe	prob. of overall trend	prob. of trend normal to mild group	prob. of trend mild to severe group
number	15	29	24			
male sex*	10(67)	20(69)	17(70)	NS	NS	NS
age <sup>†</sup> (years)	47±15	57±14	69±14	<0.0001	0.0243	0.0065
CVD*	0(0)	4(13)	9(37)	0.0083	0.1376	0.0045
DM*	3(20)	3(10)	6(24)	NS	NS	NS
hypertension*	2(13)	8(27)	20(83)	<0.0001	0.2958	<0.0001
hyperlipidaemia*	2(13)	3(10)	10(59)	0.0003	0.7736	0.0002
eGFR <sup>†</sup> (ml/min)	95±9	73±7	16±4	<0.0001	<0.0001	<0.0001
SBP <sup>†</sup> (mmHg)	141±19	146±27	156±29	NS	NS	NS
DBP <sup>†</sup> (mmHg)	81±11	84±14	77±9	NS	NS	NS
PP <sup>†</sup> (mmHg)	59±14	62±17	77±24	0.0056	0.519	0.0104
diameter <sup>†</sup> (mm)	6.9±0.9	7.0±0.8	7.2±1.1	NS	NS	NS
$Ep^{\dagger}$ (KPa)	120±48	177±104	268±174	<0.0001	0.0009	0.0102
$E^{\dagger}$ (KPa)	549±281	676±378	1091±607	<0.0001	0.0005	0.0144
IMT <sup>†</sup> (mm)	0.84±0.24	0.81±0.19	0.88±0.20	NS	NS	NS

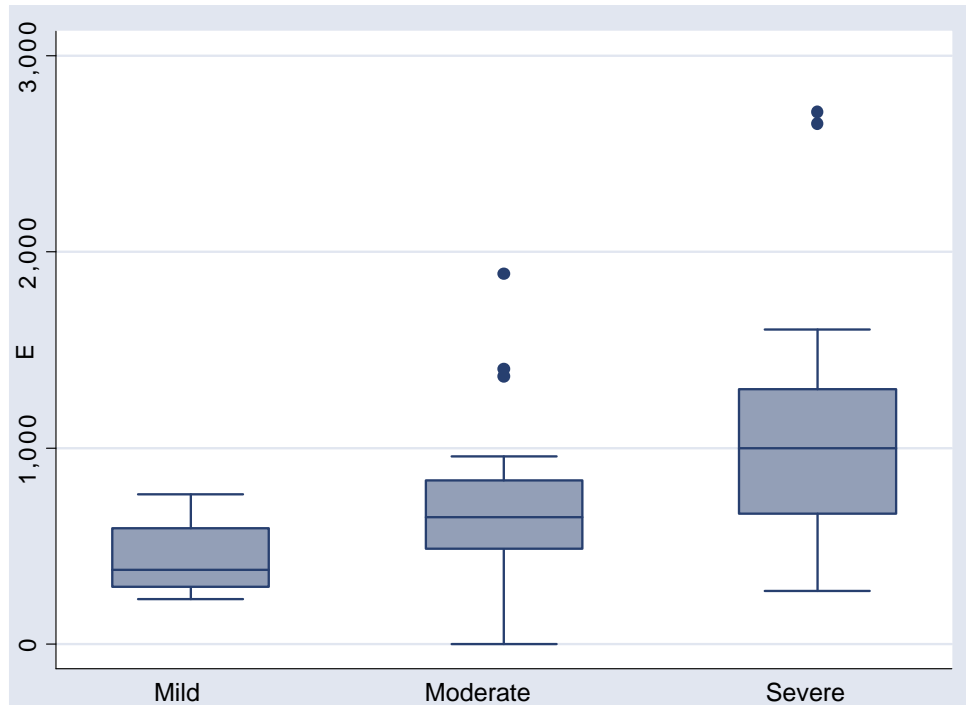
**Legend:** prob.=probability; CVD=cerebrovascular disease; DM=diabetes mellitus; GFR=glomerular filtration rate; SBP=systolic blood pressure; DBP=diastolic blood pressure; PP=pulse pressure;  $Ep$ =Peterson's elastic modulus;  $E$ =Young's modulus; IMT=intima media thickness; \*figure in brackets represents percentage per population; <sup>†</sup>values expressed as mean ± SD. Probability for trend calculated using one way ANOVA.

**Table 13:** Results of logistic regression model looking for predictors for change between Normal and Mild group and then Mild and Severe group.

<b>parameter</b>	<b>Normal to Mild</b>	<b>Mild to Severe</b>
<b>age</b>	0.008	0.001
<b>PP</b>	NS	NS
<b>IMT</b>	NS	NS
<b>E</b>	0.019	0.039
<b>Ep</b>	0.031	NS
<b>sex</b>	NS	NS
<b>diabetes</b>	NS	NS

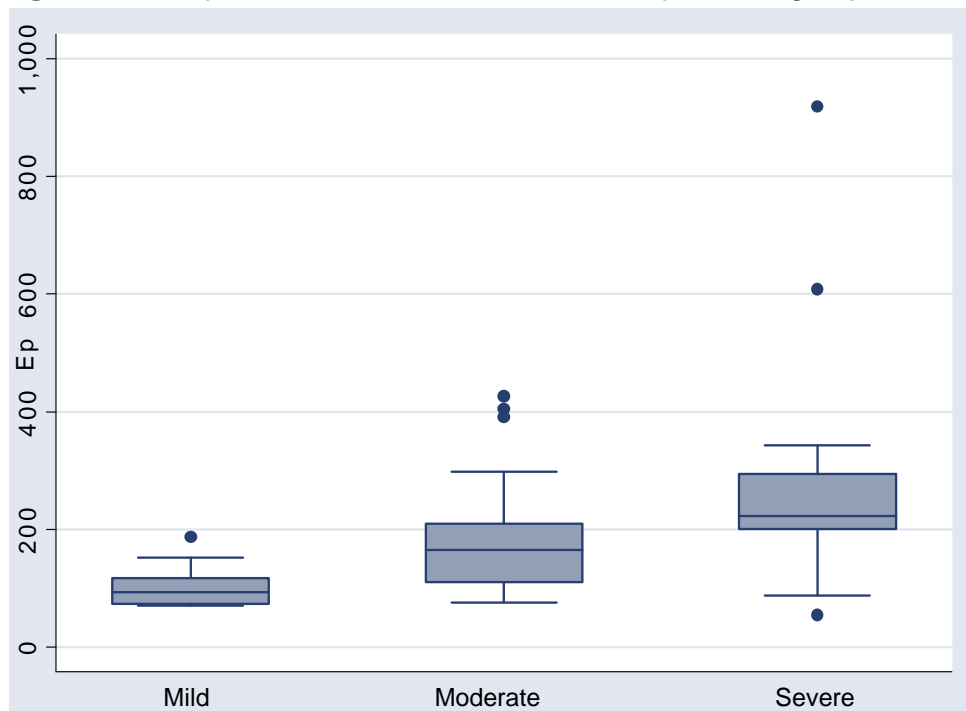
**Legend:** PP=pulse pressure; IMT=intima media thickness; E=Young's modulus; Ep=Peterson's elastic modulus; model included age, sex and diabetes. Figure indicated is p value for each variable; NS indicates p value greater than 0.05.

**Figure 25:** Box plot of Young's modulus per renal group



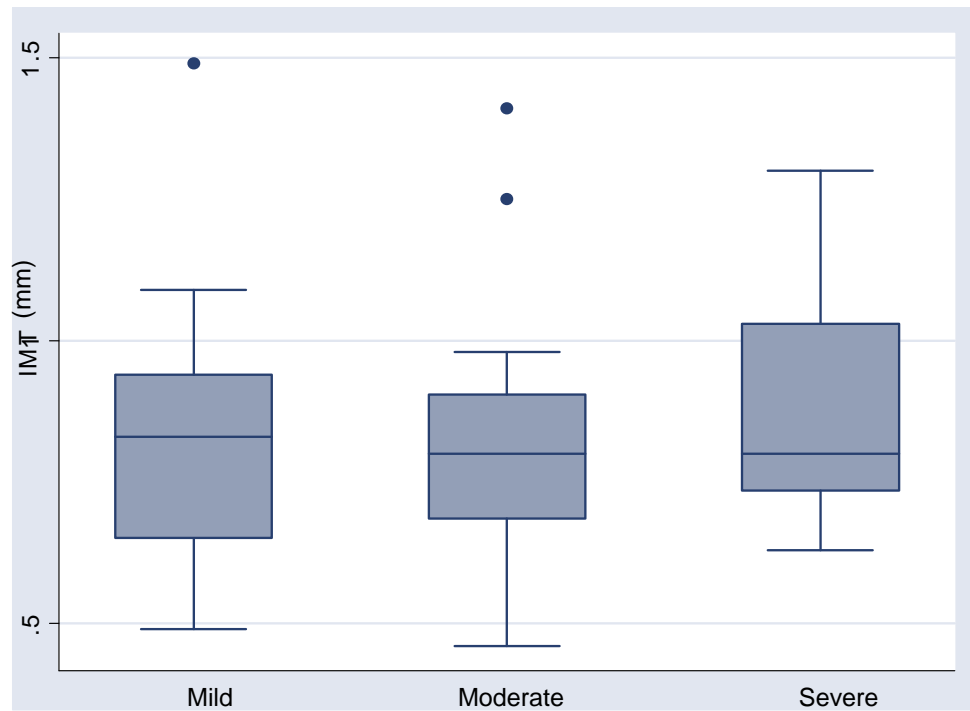
**Legend:**  $E$ =Young's modulus; renal group represented in order across x-axis, Normal, Mild, Severe; scale on y-axis in KPa.

**Figure 26:**Box plot of Peterson's elastic modulus per renal group



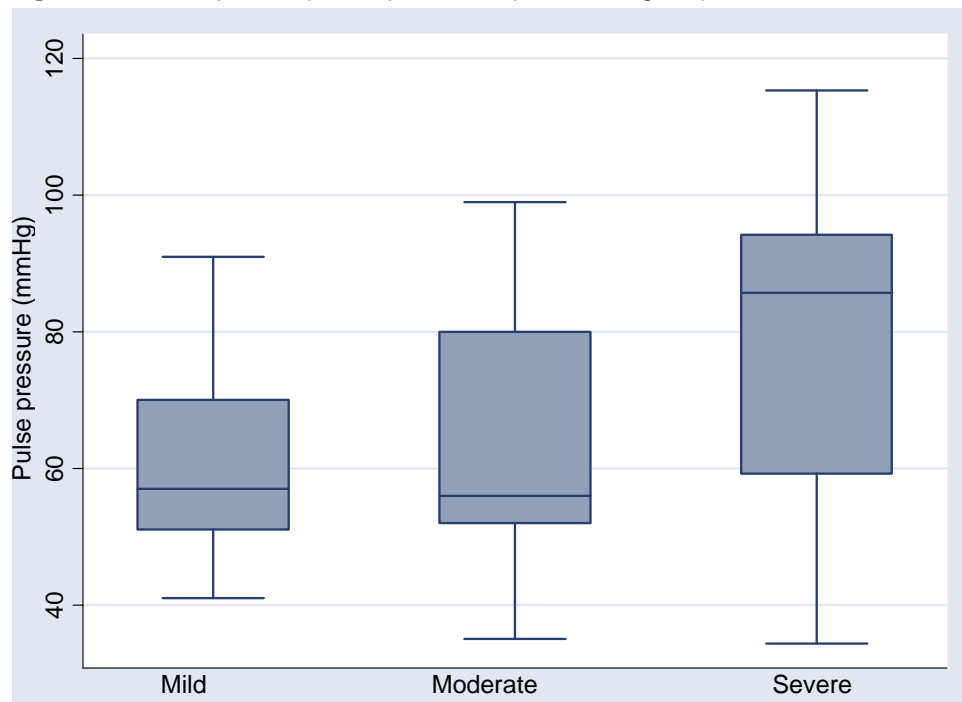
**Legend:**  $E_p$ = Peterson's elastic modulus(KPa); renal group represented in order across x-axis, Normal, Mild, Severe; scale on y-axis in KPa.

**Figure 27:** Box plot of intima media thickness per renal group



**Legend:** IMT=intima media thickness; renal group represented in order across x-axis, Normal, Mild, Severe. Scale on y-axis in mm.

**Figure 28:** Box plot of pulse pressure per renal group



**Legend:** PP=pulse pressure; renal group represented in order across x-axis, Normal, Mild, Severe. Scale on y-axis in mmHg.

## Discussion

In line with other published studies investigating the relationship between arterial stiffness and renal disease, we have shown that age, cardiovascular disease, hypertension and hyperlipidaemia as well as indices of arterial stiffness are significantly related to renal dysfunction.<sup>256</sup> However we found that the change in arterial stiffness was most marked between the Normal group and the Mild group, whereas the remainder of these parameters (with the exception of age) were not found to be significantly changed until a comparison was made with subjects with the Severe group. These findings remained significant when further statistical analysis between the Normal and Mild group was made using logistic regression. Similar analysis between the Mild and Severe group suggested that only age and  $E$  was significantly associated.

The difference in  $E$  and  $E_p$  between the Normal and Mild group suggests that there is an increase in both the material and overall structural stiffness of the arterial wall early in the development of renal disease. This may occur before other markers of cardiovascular disease are apparent such as increased pulse pressure or development of increased IMT or arterial diameter. This may be because the observed increase in arterial wall stiffness in subjects with renal disease occurs due to different mechanisms from that found in a normal population. Putative mechanisms include changes in the quantity<sup>30</sup> and elasticity<sup>181</sup> of extracellular collagen together with medial calcification.<sup>182</sup>

Increased Carotid IMT secondary to hypertension and wall stress has been noted in CKD patients,<sup>30</sup> but this may be a late change, as we did not find any significant increase in this in our study. It is interesting that the statistically significant difference in  $Ep$  but not  $E$  was lost in our regression model looking at the Mild and Severe group, as this suggests continued change in the material stiffness of the arterial wall as CKD progresses, but not a resultant change in overall structural stiffness.

The numbers involved in this retrospective study are small and our findings need validation via a larger prospective study. In particular this would permit further regression analysis with a more complex model than the one used in our study (age, sex and diabetes). In addition we recruited three distinct groups of subjects with normal renal function, mild renal impairment and severe renal impairment. Thus was a flaw in our study design as this study would have yielded more information if the study population included subjects with all CKD stages and thus a full spectrum of eGFRs. We did not include any subjects receiving renal replacement therapy as we believed it likely that dialysis would cause too much flux in data collection to be valid.

## Conclusions

This study has shown a statistically significant increase in both  $E$  and  $Ep$  when comparing healthy subjects with mild renal disease. This was not the case for IMT or PP. This finding was maintained for  $Ep$  but not  $E$  in a comparison of subjects with mild and severe renal disease, suggesting

that there is continued change in material arterial wall stiffness as CKD progresses although this may not manifest as increased overall structural stiffness.

We believe that therapies to target the early change in wall stiffness may be the key to diminishing the high prevalence of cardiovascular disease found in subjects with CKD, as it may be too late in the disease process to attempt to reverse in reaction to other changes such as increased IMT and PP and still expect a significant decrease in the prevalence of cardiovascular disease in subjects with CKD.

## ACE INHIBITORS INCREASE TYPE III COLLAGEN SYNTHESIS: A POTENTIAL EXPLANATION FOR REDUCTION IN ACUTE VASCULAR EVENTS BY ACE INHIBITORS

*Large trials have shown that Angiotensin Converting Enzyme Inhibitor (ACE-I) therapy reduces the risk of myocardial infarction and stroke. Acute vascular events are thought to be initiated by plaque rupture. Animal models of atherosclerosis show an increase in extra cellular matrix when given ACE-I therapy. ACE-I therapy could influence collagen synthesis, one of the major constituents of the atherosclerotic cap.*

*Subjects were assessed for arterial disease, drug history and smoking. Blood samples were taken for a measure of collagen synthesis, the amino-terminal propeptide of type III procollagen (PIIINP), lipid levels, iron metabolism and cotinine levels. Information was available for 420 subjects. 35 were taking ACE-I therapy and 385 were not. Mean serum PIIINP level was 3.5 µg/l (SD 1.3 µg/l, range: 1.7 µg/l to 16.5 µg/l). There was a marked increase in mean collagen turnover between subjects taking ACE-I therapy compared to those not. Mean PIIINP level for cases and controls was 4.26 µg/l (95% CI: 3.73 – 4.79 µg/l) versus 3.61 µg/l (95% CI: 3.48 – 3.75 µg/l). After adjusting for age, weight, height, lipid levels and ferritin, PIIINP levels remained significantly higher in cases than controls: 4.14 µg/l (95% CI: 3.72 – 4.57 µg/l) versus 3.62 µg/l (95% CI: 3.49 – 3.75 µg/l) (P value 0.02).*

*These results suggest that ACE-I therapy up regulates collagen synthesis, and could improve plaque stabilisation. This may provide an explanation for the decrease in acute vascular events observed in patients on ACE-I therapy.*



## Introduction

Several large trials have shown that treatment with ACE inhibitors significantly reduces the risk of myocardial infarction and stroke. This effect was demonstrated to be independent of reduction in blood pressure and concomitant medication.<sup>262</sup> It is thought that most acute vascular events begin with rupture of an atherosclerotic plaque. This suggests that ACE inhibitors lower the risk of atherosclerotic plaque rupture.

Unstable plaques, at higher risk of rupture, tend to have a thin, friable fibrous cap. The cap consists of extra cellular matrix, the chief component of which is collagen. Type I and III are the major structural components that confer tensile strength to the fibrous cap.<sup>263,264</sup> Type III is also the major component of large arteries, muscle and skin.<sup>265,266</sup> Collagen homeostasis is determined by the opposing metabolic processes of collagen synthesis and collagen degradation. A large amount of research has implicated increased matrix metalloproteinase (MMP) activity as an important factor in increased collagen degradation.<sup>267-269</sup> However, it is important to consider increased collagen synthesis as well. This study was performed to investigate the effect of ACE-Is on collagen type III synthesis.

The hypothesis was that subjects taking ACE-Is would have increased collagen type III synthesis when compared to a matched control population.

## Methods

### *Subject selection*

A screening programme for asymptomatic abdominal aortic aneurysms in males over the age of 50 started in Huntingdon, England in November 1991. The cohort of screened men over the age of 50 living in the Huntingdon District served as a basis for this study. All subjects underwent informed consent, and ethical approval was gained locally. All subjects were interviewed about their family history, previous medical history, drug history, and smoking habits. All subjects had a brief medical examination by the same observer. The medical examination included measurement of the ABPI and the aortic diameter by ultrasound. PAD was deemed present if the ABPI was less than 0.9. An aneurysm was defined as an infrarenal aortic diameter of 3 cm or more.

A blood sample was taken from all patients for measurement of serum collagen turnover, lipid levels, markers of iron metabolism and cotinine levels. Smoking and cholesterol are standard risk factors for atherosclerotic disease and acute vascular events. Therefore measurements of serum cotinine levels and lipid levels allowed us to adjust for possible differences in the distribution of those risk factors between the groups exposed to ACE inhibitors and those not exposed. Markers of iron metabolism were measured as iron is an important cofactor in collagen metabolism.<sup>265,266</sup> These markers consisted of serum iron, transferrin, ferritin and antichymotrypsin levels.

Blood samples were taken at the time of the medical examination. They were spun down and the serum frozen at -21°C on the same day for analysis later. Batches of 74 serum samples were analysed in duplicate on the same day, by the same biochemist from the Department of Chemical Pathology in Hinchingsbrook Hospital. The concentration of the amino-terminal propeptide of type III procollagen (PIIINP) was used as a proxy measure of type III collagen turnover. The amino-terminal propeptide is split from the procollagen molecule during type III collagen synthesis.<sup>265,266</sup> The concentration of PIIINP was measured using an equilibrium type serum radio-immunoassay for the amino-terminal propeptide of type III procollagen. This was based on highly purified specific human antigen (Pharmacia & Upjohn Ltd, Diagnostics Division, Milton Keynes, UK) and performed according to the instructions of the manufacturer with the use of duplicate 200 µL aliquots of serum.<sup>270,271</sup> The assay detects the authentic amino-terminal propeptide of type III collagen, but is not sensitive to the smaller degradation products of the propeptide.<sup>270</sup> The antigens are resistant to repeated thawing and freezing.<sup>271</sup> The benefit of this assay is that standard and human serum samples give parallel inhibition curves allowing more accurate reading of serum levels without the need for repeated dilution and analysis.<sup>270</sup> The intra-assay and inter-assay coefficients of variation of this assay are less than 5% and the sensitivity is 0.2 µg/L. The reference range for adults, based on data of Finnish blood donors was 1.7 to 4.2 µg/L, with no differences between males and females.<sup>270</sup> The lipid profile consisting of

serum total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides was determined using an automated procedure on a random blood sample. Blood samples were taken during clinic attendance. Screening clinics were in the evening at 5pm or on Saturday morning. No instructions about food intake were given prior to the medical examination. Lipid profiles were measured within a few days in the Department of Chemical Pathology at Hinchingsbrooke Hospital, as part of their standard routine lipid assays. Total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides were measured using standard enzymatic methods in a fully automated procedure using a Roche Hitachi 717 analyser with Bio Stat reagents. Serum cotinine levels were measured using a standard semi-quantitative microplate enzyme immunoassay (Cozart Biosciences, Abingdon, UK).

## **Statistical Analysis**

Mean values were compared using a t-test. Multivariate analysis was performed using linear regression methods<sup>272</sup> with STATA 5.0 for Macintosh.<sup>273</sup> Adjusted mean PIIINP levels were calculated using logistic regression models.<sup>274</sup> Adjusted means were compared using Fisher's F test.

## Results

Assessment of collagen turnover in the form of PIIINP levels was available for 456 subjects. Detailed information about medication and PIIINP levels were available for 420 subjects. Of these 420 subjects, 180 had no evidence of PAD and no aneurysmal disease, 34 had evidence of PAD but no aneurysmal disease, 129 had a small aneurysm (30 - 45mm diameter) but no evidence of PAD, 60 patients had both a small aneurysm and PAD and in 17 patients data about PAD were missing. They were included in the study. 35 subjects were on ACE inhibitors and 385 were not. Mean serum PIIINP level was 3.5 µg/l (SD 1.3 µg/l). A large variation in PIIINP levels was found, range: 1.7 µg/l to 16.5 µg/l. There was a significant increase in mean collagen turnover between subjects on ACE inhibitors compared to those not on ACE inhibitors. Mean PIIINP levels for subject on ACE inhibitors was 4.26 µg/l (95% CI: 3.73 – 4.79 µg/l), compared to 3.61 µg/l (95% CI: 3.48 – 3.75 µg/l) for subjects not exposed to ACE inhibitors. Mean PIIINP levels of the 36 subjects with missing data about current medication was 3.78 µg/l (95% CI: 3.30 – 4.27 µg/l). There were no statistically significant differences in type III collagen turnover between subjects on other anti-hypertensive drugs such as: calcium channel blockers, diuretics or betablockers (see Table 14). There were no significant differences in PIIINP levels between subjects with a small aneurysm (mean PIIINP: 3.73 µg/L 95% CI 3.53 – 3.93) and subjects with a normal aorta (mean PIIINP: 3.63 µg/L 95% CI 3.47 – 3.79)

(P value .46). There were no significant differences in PIIINP levels between subjects with evidence of PAD (mean PIIINP: 3.71 95% CI 3.47: – 3.95) and subjects with no evidence of PAOD (mean PIIINP: 3.65µg/L 95% CI: 3.50 – 3.80) (P value .71). The difference in collagen turnover remained significant after adjusting for presence of aneurysmal disease or evidence of PAOD. Adjusted mean PIIINP for subjects on ACE inhibitors was 4.27 µg/L, (95% CI 3.83 – 4.71 compared to a mean PIIINP of 3.59 µg/L, (95% CI 3.46 – 3.73 for those not on ACE inhibitors (P value .0046).

Stepwise regression was used to investigate which other variables were associated with PIIINP levels. Variables investigated were age, height, weight, systolic and diastolic blood pressure, diabetic status, smoking status in the form of serum cotinine levels, and the usual standard lipid risk factors such as: total cholesterol levels, HDL cholesterol, LDL cholesterol and triglycerides. Iron is an important cofactor of collagen metabolism. We also investigated the following measurements of iron metabolism: serum iron levels, transferrin levels, ferritin levels and antichymotrypsine. Stepwise regression showed that PIIINP levels were significantly associated with age, height, weight, diastolic blood pressure and ferritin levels. None of these were significantly different between subjects on ACE inhibitors and those not on ACE inhibitors (see Table 15). We calculated adjusted mean PIIINP levels between subjects exposed to ACE inhibitors and those not exposed. The difference in mean PIIINP levels remained significant after

adjusting for age, height, weight, HDL and ferritin: 4.14 mcg/l (95% CI: 3.72 – 4.57 mcg/l) for subjects exposed to ACE inhibitors versus 3.62 mcg/l (95% CI: 3.49 –3.75 mcg/l) (P value 0.02).

**Table 14:** Collagen turnover as measured by serum PIIINP concentration between subjects exposed to classes of anti hypertensive drugs and those not exposed.

drug	number exposed	mean PIIINP	number not exposed	mean PIIINP	P value
ACE -I	35	4.26 (3.73 - 4.79)	378	3.62 (3.49 - 3.76)	0.007
Ca channel	45	3.53 (3.25 - 3.82)	368	3.70 (3.56 - 3.84)	0.65
diuretics	64	3.95 (3.61 - 4.30)	349	3.63 (3.49 - 3.77)	0.08
betablockers	77	3.80 (3.52 - 4.08)	336	3.65 (3.51 - 3.80)	0.25

**Legend:** PIIINP levels in mcg/L, with 95% confidence levels in brackets; p values were calculated by applying the t-test to the log transformation of the mean PIIINP between subjects exposed to classes of anti hypertensive drugs and those not exposed.

**Table 15:** Confounding variables associated with PIIINP levels and the differences between subjects on ACE-I and those not.

variable	on ACE-I	not on ACE-I	P value
age	70.7 (68.5 – 73.1)	70.8 (70.1 – 71.6)	0.95
height (cm)	172.1 (170.1 – 174.1)	173.5 (172.8 – 174.2)	0.24
weight (kg)	81.6 (76.9 – 86.3)	80.1 (79.0 – 81.3)	0.49
HDL (mmol/l)	1.05 (0.95 – 1.14)	1.16 (1.11 – 1.21)	0.20
Ferritin (mcg/l)	136.9 (101.1 – 172.8)	118.4 (108.4 – 128.3)	0.29

**Legend:** HDL=high density lipoprotein cholesterol; figures in brackets are 95% confidence intervals

## Discussion

A marked increased type III collagen synthesis, as measured by PIIINP levels, was seen in subjects on ACE-Is compared to those not exposed to ACE-Is. The difference was highly significant and remained significant after adjusting for confounding variables.

Marshall et al<sup>275</sup> demonstrated a reduction in collagen metabolism in lung tissue after administration of intratracheal Ramipril (an ACE-I) and Losartan (an Angiotensin II type 1 antagonist). This finding was also noted in their control population where intratracheal saline was administered. There was also clear evidence that ACE was not the only controlling factor for Angiotensin II expression in this experiment.

Another in vitro experiment<sup>276</sup>, this time utilising rat cardiac cells, investigated the role of N-Acetyl-Seryl-Aspartyl-Proline (Ac-SDKP), a natural inhibitor of stem cell entry into S phase (the period for collagen synthesis). This molecule is hydrolysed by ACE-Is. They found it to have a biphasic and dose dependant effect on collagen synthesis. In low doses it had an inhibitory effect on collagen synthesis, but in high dose no significant effect was noted.

The use of ACE-Is in other animal models of atherosclerosis has shown a decrease in plaque area with a decrease in macrophage accumulation. An increase in the extracellular matrix was also demonstrated.<sup>277</sup> These phenomena could be explained by the up



regulation of collagen synthesis, and the presence of increased quantities of collagen in the fibrous cap.

Reduced collagen content in the fibrous cap may result from decreased synthesis of the extra-cellular matrix by VSMCs, increased breakdown, or both. VSMCs synthesize the precursors of type III collagen for the extracellular matrix. The synthesis and degradation of the extracellular matrix proteins are slow in the normal artery. Atherosclerosis and arterial injury lead to increased synthesis of many matrix components including collagen type III,<sup>277</sup> presumably as part of the response to the insult.

## **Conclusions**

These results suggest that ACE-I therapy may increase collagen synthesis, and thus plaque stabilisation. This may provide an alternative explanation for the decrease in acute vascular events observed in patients on ACE-I therapy.

## ASSESSMENT OF CAROTID ARTERY STIFFNESS AND COLLAGEN TURNOVER IN SUBJECTS WITH PERIPHERAL ARTERIAL DISEASE COMMENCED ON RAMIPRIL.

*ACE-Is cause a reduction in secondary cardiovascular events, independent of blood pressure. This may be secondary to vascular remodelling. In chapter 12 we have demonstrated that ACE-I administration in subjects with PAD is associated with increased collagen turnover. The aim of this chapter was to carry out a longitudinal study into the effects of ACE-I administration in subjects with PAD.*

*33 subjects with confirmed PAD were recruited into the study, and were commenced on Ramipril. PIIINP, BP, IMT, d, E and Ep were measured at baseline, 12week, 26weeks and 52 weeks. 13 completed the entire study. At 52 weeks, PIIINP levels were significantly higher (2.14 versus 2.67  $p=0.018$ ), together with a significant decrease in SBP (159 versus 142 mmHg  $p=0.011$ ), IMT (1.24 versus 0.93 mm  $p=0.003$ ) and diameter (8.51 versus 6.90mm  $p=0.013$ ). Ep was significantly less at 26 weeks but not at 52 weeks, and E was significantly less at 12 weeks but not thereafter.*

*We have shown statistically increased collagen turnover as estimated by PIIINP levels in subjects with PAD commenced on Ramipril. However the subject population was small with a high dropout rate. Therefore whilst the results may not be valid, they confirm our hypothesis of chapter 12 and warrant further study.*

## Introduction

The significant reduction in secondary cardiovascular events seen in subjects taking Ramipril who have cardiovascular disease is well recognised from large epidemiological studies.<sup>262</sup> This effect is independent of blood pressure reduction and may well be secondary to vascular remodelling and plaque stabilisation, together with reduction of large artery stiffness.<sup>159</sup> The RAAS has long been implicated in subjects with arterial hypertrophy secondary to hypertension. Increased lumen size, IMT, collagen deposition and decreased compliance have all been noted.<sup>278-280</sup> Collagen homoeostasis may be influenced by inhibition of MMPs on the basis that ACE-Is bind zinc, a cofactor for a number of MMPs.<sup>281</sup> Using the amino terminal pro-peptide of type III procollagen (PIIINP) as a marker of collagen turnover, previous results suggest that collagen synthesis is increased in subjects taking ACE-Is (see chapter 12). However we recognize that this finding needs to be confirmed in a prospective study. Ahimastos et al have shown decreased arterial stiffness in a case control study subjects with PAD given Ramipril as opposed to placebo. They also demonstrated that Ramipril promoted elastogenic remodelling in cell cultures.<sup>159</sup> Decreased arterial stiffness from baseline in subjects commenced on Ramipril would provide further evidence to support the presence of non anti-hypertensive beneficial effects of ACE-Is.

The aim of this study was to measure collagen turnover and arterial stiffness in a cohort of subjects with PAD who were commenced on Ramipril.

The hypothesis was that collagen synthesis would increase and that arterial stiffness would decrease over time in these subjects when commenced on Ramipril.

## **Methods**

### ***Subject Selection***

Ethical approval was obtained for this study from the East Birmingham Research and Ethics Committee. Subjects were recruited from the vascular surgical outpatients clinics of Heart of England NHS foundation trust and provided written informed consent. All subjects had a confirmed diagnosis of PAD based on history and ABPI of less than 0.9 in at least one leg. Patients were excluded from the study if already taking an ACE-I or Angiotensin II receptor blocker (A2RB), had abnormal renal function or a history of renal artery stenosis (RAS). Subjects entered into the study were commenced on Ramipril starting at 2.5 mg for one week, 5 mg for three weeks and increased to the maintenance dose of 10 mg thereafter. Arterial stiffness and collagen turnover was assessed at baseline, at 12 weeks, 26 weeks and 52 weeks.

### ***Measurement of collagen turnover***

Blood samples were taken at the specified intervals. They were spun down and the serum frozen at -21°C on the same day for subsequent analysis. The serum samples were analysed in duplicate on the same day, by a single biochemist from the Department of Pathology at the Heart of England NHS Foundation Trust. The method used was exactly the same as that described on page 118.

### ***Statistical analysis***

After extraction and collation of the raw data using Microsoft Excel, the data were analysed using Stata 8.1 for Windows (STATA Stata Corporation, College Station, Texas, USA). Measurements at 12, 26 and 52 weeks of arterial stiffness indices, PIIINP, IMT, diameter and blood pressure were examined for significant differences with baseline measurements using Wilcoxon signed ranks.

## **Results**

33 subjects were recruited into the study. However 20 of the subjects were unable to complete the trial period, either being non compliant with the study drug or being lost to follow-up. The main demographics are demonstrated in Table 16, pre-existing medication in Table 17. At 52 weeks, PIIINP levels were significantly higher (2.14 versus 2.67  $p=0.018$ ), together with a significant decrease in SBP (159 versus 142

mmHg  $p=0.011$ ), IMT (1.24 versus 0.93 mm  $p=0.003$ ) and diameter (8.51 versus 6.90mm  $p=0.013$ ).  $E_p$  was found to be significantly less at 26 weeks but not at 52 weeks, and  $E$  was found to be significantly less at 12 weeks but not thereafter (Table 18). Figures 29-34 show box plots of all of these variables.

**Table 16:** main demographics of ARC Study population

variable	age	male	smoking	HTN	CAD	CVD	DM
	68 (51-84)	39 (76)	29 (57)	28 (55)	4 (8)	1 (2)	8 (16)

**Legend** HTN = hypertension; CAD = coronary artery disease; CVD = cerebrovascular disease; DM = diabetes mellitus; figure in brackets is percentage of prevalence in whole study population except for age where figure is range in years.

**Table 17:** drug history for ARC study population

drug	Statin	diuretic	ca antagonist	nitrate	b-blocker
	43 (84)	8 (16)	9 (18)	1 (2)	3 (6)

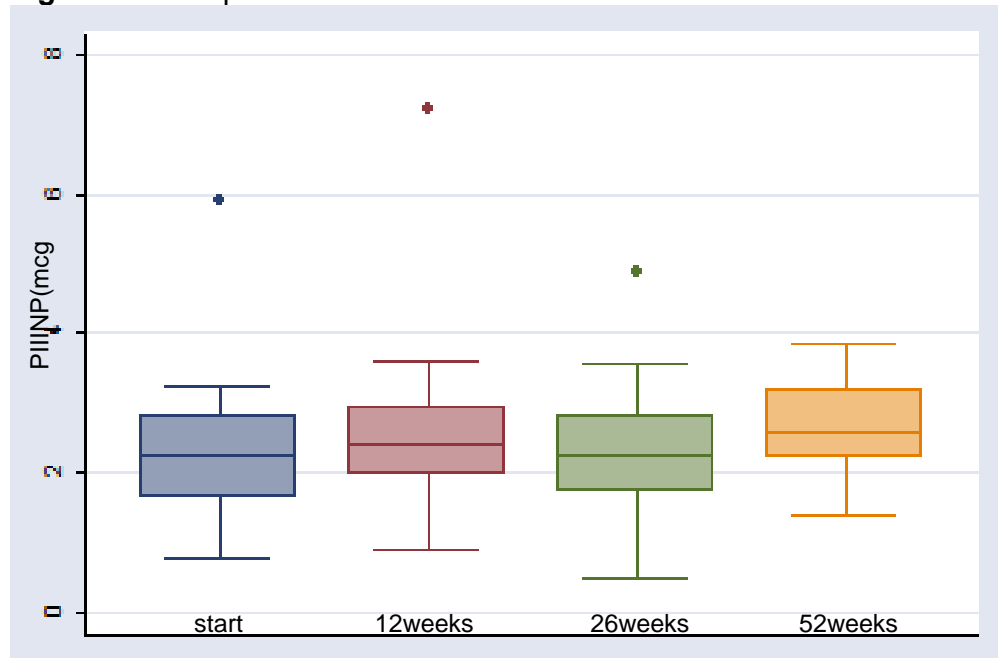
**Legend:** figure in brackets is percentage of prevalence in whole study population.

**Table 18:** Mean values for parameters in ARC study population.

variable	start	12wks	26wks	52wks
<b>no of subjects</b>	33	23	20	13
<b>PIIINP</b>	2.41 (0.76-5.90)	2.62 (0.53-7.24) <i>0.112</i>	2.36 (0.46-4.87) <i>0.724</i>	2.65 (1.38-3.83) <i>0.050</i>
<b>SBP</b>	152 (106-203)	145 (113-194) <i>0.002</i>	139 (116-168) <i>0.000</i>	142 (134-149) <i>0.000</i>
<b>IMT</b>	1.13 (0.66-1.83)	1.07 (0.71-1.56) <i>0.000</i>	0.87 (0.30-1.44) <i>0.000</i>	0.92 (0.78-1.11) <i>0.000</i>
<b>d</b>	8.38 (5.58-10.54)	7.67 (6.00-10.00) <i>0.000</i>	7.11 (5.14-10.04) <i>0.000</i>	7.08 (5.40-8.55) <i>0.000</i>
<b>Ep</b>	324 (75-2309)	231 (63-1314) <i>0.007</i>	147 (87-245) <i>0.001</i>	185 (98-342) <i>0.004</i>
<b>E</b>	1301 (251-7425)	3925 (1092-15237) <i>0.000</i>	684 (234-2034) <i>0.001</i>	714 (349-1481) <i>0.001</i>

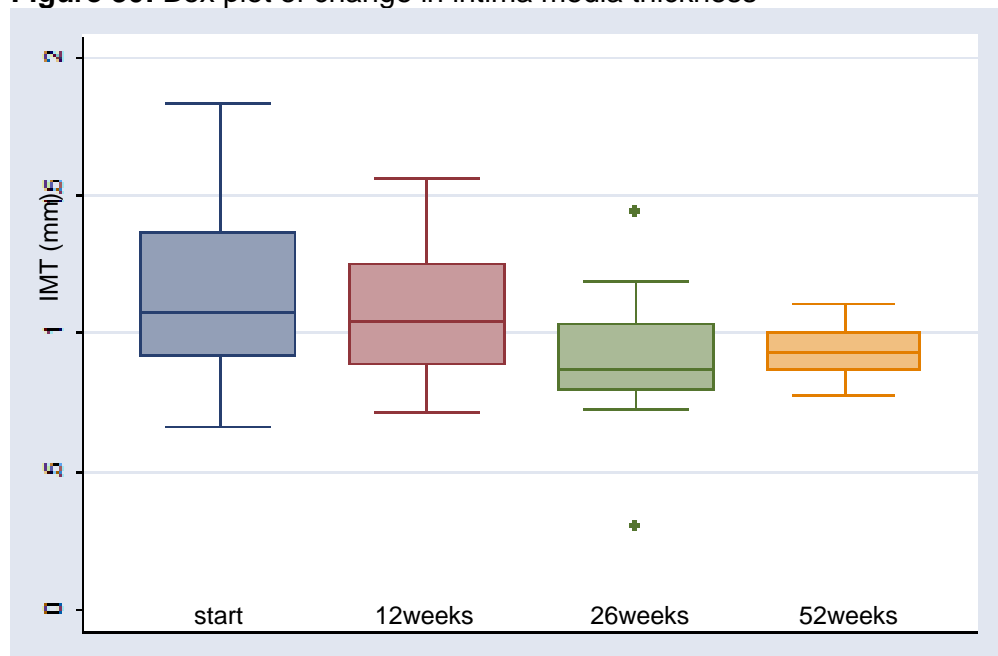
**Legend:** PIIINP = amino terminal pro-peptide of type III procollagen; SBP = systolic blood pressure; IMT = intima media thickness; d = diameter; Ep = elastic modulus; E = Young's modulus; figures in brackets are 95% confidence intervals; figure in italics is p value comparing parameter with starting value.

**Figure 29:** Box plot of PIIINP levels



**Legend:** blue= results at start; red= 12weeks; green= 26weeks; yellow= 52weeks; y axis PIIINP (mcg/L)

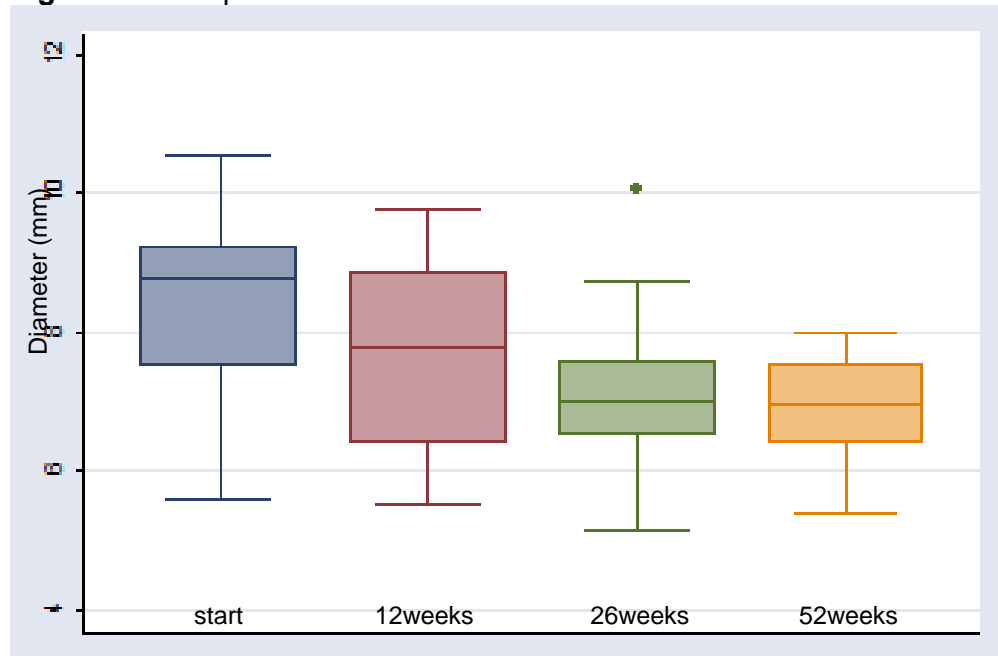
**Figure 30:** Box plot of change in intima media thickness



**Legend:** blue= results at start; red= 12weeks; green= 26weeks; yellow= 52weeks; y axis IMT (mm)

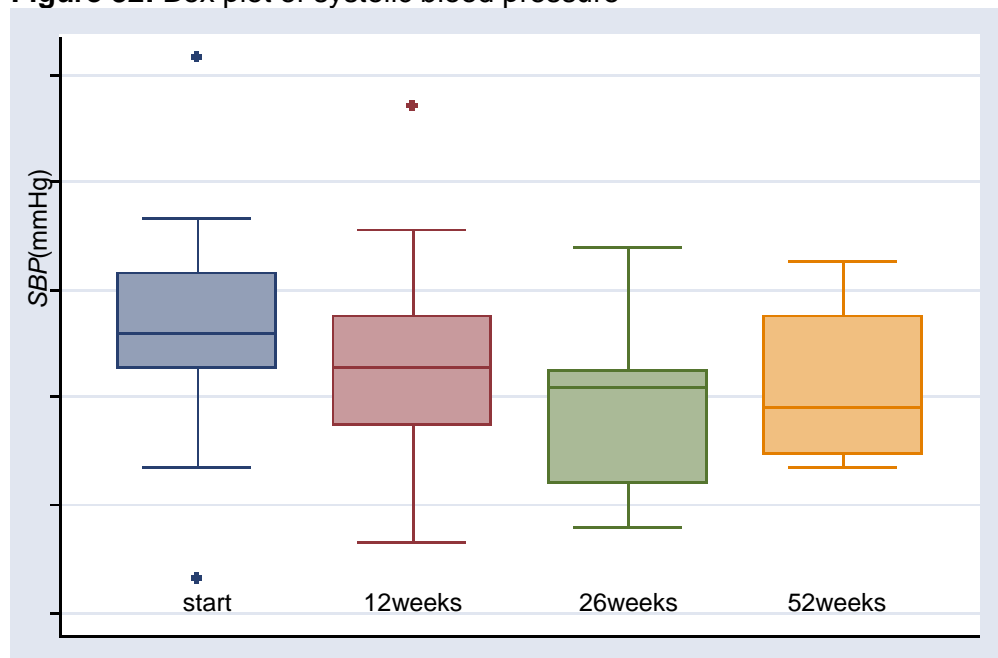


**Figure 31: Box plot of diameter**



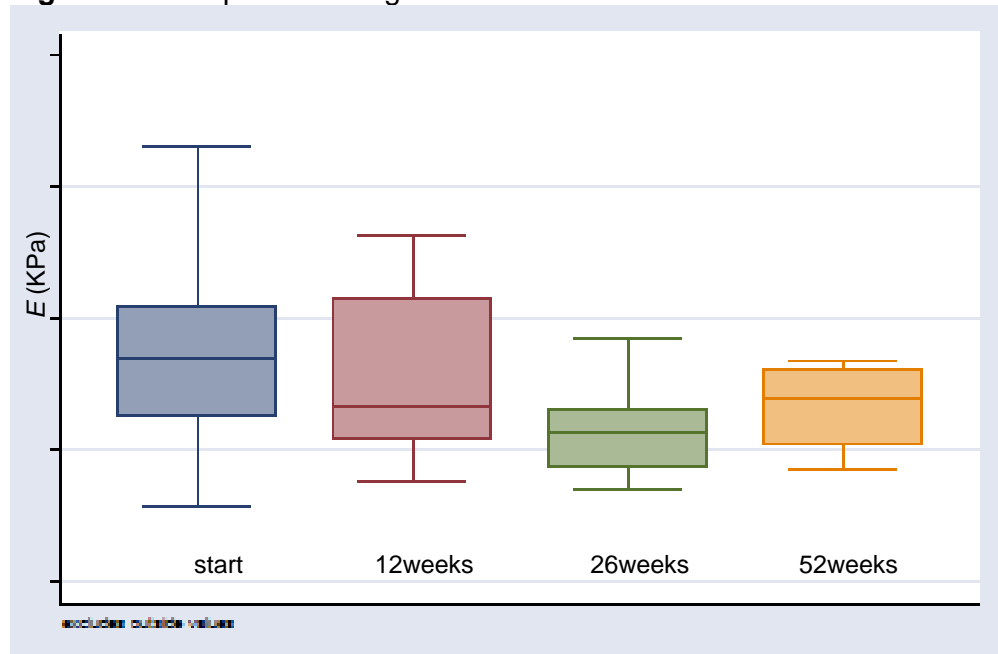
**Legend:** blue= results at start; red= 12weeks; green= 26weeks; yellow= 52weeks; y axis diameter (mm)

**Figure 32: Box plot of systolic blood pressure**



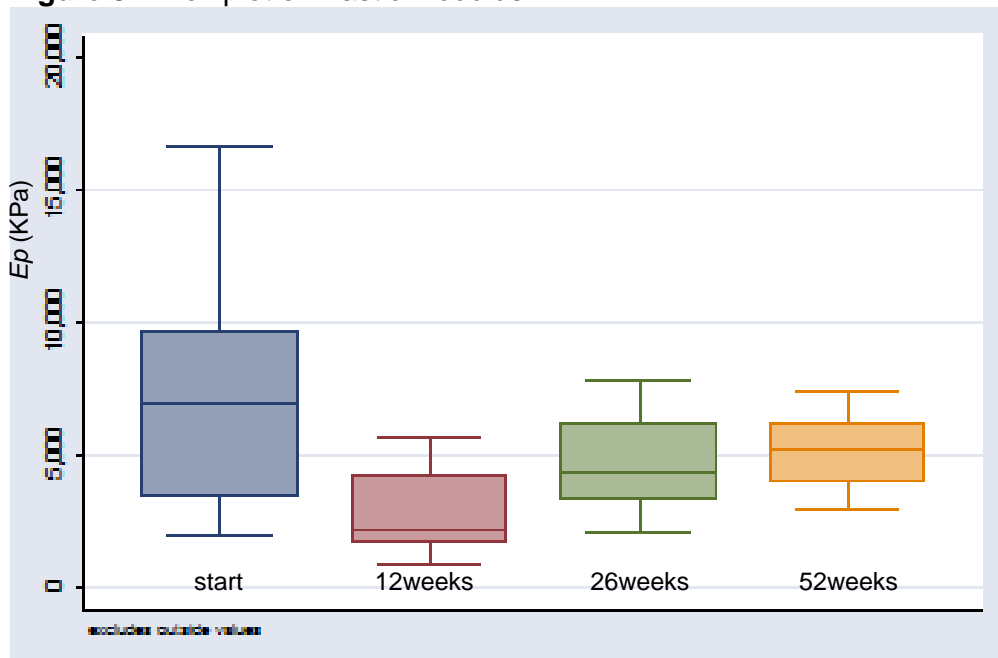
**Legend:** blue= results at start; red= 12weeks; green= 26weeks; yellow= 52weeks; y axis SBP (mmHg)

**Figure 33: Box plot of Young's modulus**



**Legend:** blue= results at start; red= 12weeks; green= 26weeks; yellow= 52weeks; y axis E (kPa)

**Figure 34: Box plot of Elastic modulus**



**Legend:** blue= results at start; red= 12weeks; green= 26weeks; yellow= 52weeks; y axis E (kPa)

## Discussion

We have shown in a cohort of subjects with PAD that administration of Ramipril results in a statistically significant increase in PIIINP turnover and a statistically significant decrease in SBP, IMT and diameter. Both  $E$  and  $Ep$  demonstrated statistically significant transient decreases during the one year follow-up period, with  $Ep$  significantly decreased at 26 weeks and  $E$  significantly decreased at 12 weeks.

Initially we intended to recruit over 50 subjects into the study, but we were only able to recruit 33 in the allocated study period (18 months) mainly due to the lack of perceived benefit from starting Ramipril in the recruitment population and the requirement to visit the research facilities separately from other clinic visits in order to have ultrasound scans and blood sampling. These factors also resulted in high dropout of the initial recruitment population such that only 13 (39%) of the subjects initially enrolled into the study were compliant with the study drug and attended follow-up. Interestingly, few subjects cited side-effects as the reason for the lack of compliance with the study drug. As a result we believe that the sample population is too small to draw any meaningful conclusions or to accept that statistical analysis outlined in the results section as the probability of a type 2 statistical error is very high. We still believe that there is merit in investigating arterial stiffness parameters in subjects with PAD commenced on Ramipril, but the size and the duration of the study would need to be increased together with increased convenience of data

collection so that this study population which is likely to be attending outpatient clinics on a regular basis is not further inconvenienced by extra visits.

The crossover study design which was used, whilst well recognised as a method to investigate the effect of a treatment on subjects with chronic disease, clearly has limitations. This study could have been designed using a placebo and intention to treat analysis. It would have been more difficult to set up and would require a full dataset for analysis but could potentially have overcome some of the limitations of the crossover design.

## **Conclusions**

Whilst we have shown statistically increased of collagen turnover as estimated by PIIINP levels in subjects with PAD commenced on Ramipril, the size of the initial subject population was small. Together with poor compliance in terms of both follow up attendance and drug administration, we are not able to draw any meaningful conclusions from these results. However these preliminary results confirm our hypothesis of chapter 12 and warrant further study.

## GENERAL DISCUSSION

The central hypotheses of this thesis are that the TDI system is able to provide a high resolution plot of the ADW that has acceptable reproducibility when calculating indices of arterial stiffness. This should then permit a detailed and accurate analysis of indices of material and structural arterial wall stiffness to show changes in these indices in subjects with increasing age, PAD, CKD and subjects with PAD commenced on Ramipril, when compared to healthy controls. I believe that I have demonstrated this to be the case in the preceding chapters.

It took a long time to develop the technique to be able to reliably capture the ADW. There were two main reasons for this. Initially the offline software analysis of the cineloops containing TDI data produced unreliable and confusingly inaccurate results that suggested at times that the system might have poor reproducibility and at other times that it might not work at all (for example failing to identify any arterial wall movement at all when this was clearly seen by the naked eye on the cineloop).

Trial and error suggested that the main reason for this was movement of the ultrasound probe head which resulted in poor scan plane alignment while cineloops were captured. Thus the maximum excursion of the arterial wall during the arterial distension waveform was not captured along the entire length of the region of interest (ROI) of the

insonated artery. As a result there was disparity in the ADW both along the ROI and from cardiac cycle to cardiac cycle. I first saw the TDI system working in a lab based setting, with the probe held in clamp and the target an arterial phantom. This error was not present in the phantom based model and at first I believed that the disparity was due to biological variation in the ADW as opposed to poor insonation. This was particularly the case given that the second investigator had over eight years experience in ultrasound and the cineloops that he captured were of similar quality to mine (I had very little experience in ultrasound before starting research). It was only when we achieved scan plane alignment by ensuring that the intima-media complex was uniformly visible across the ROI during the whole time of cineloop capture that we started to get meaningful and reproducible results. Achieving scan plane alignment is a basic requirement of good sonographic technique. Most dynamic velocity measurements using ultrasound in clinical practice provide immediate feedback of change in scan plane alignment via loss or dampening of the waveform. This feedback loop is not present in the TDI system and thus it is perhaps not surprising that this requirement took time to be identified as source of error together with finding a method reliably ensuring scan plane alignment for the entire duration of cineloop capture.

Once reliable cineloops could be collected, it quickly became apparent that the TDI system generated a huge amount of data (Appendix 2) which was very time consuming to analyse until the analysis macro was written to speed this process up (Appendix 2). It was only at

this point that we were able to start investigating the potential of the TDI system.

We chose to assess arterial stiffness using Peterson's elastic modulus and Young's modulus. These two indices were chosen after discussion with a medical physicist as they are the most widely accepted informative measures of wall stiffness, both within the field of medical physics and in the wider field of engineering as a whole. A major benefit of assessing arterial stiffness using a system such as the TDI based method is that it permits measurement of IMT and thus calculation of Young's modulus. As previously discussed, there is discrepancy in the published literature as to the correct formula for Young's modulus. We worked back from first principles, with a medical physicist, in order to be sure which was the correct formula to use to analyse and interpret our results (Appendix 1, page 144).

Assessment of arterial stiffness is based on elastic theory, which requires that the observed structure (in this case the arterial wall) is perfectly elastic and constructed from a homogenous and isotropic material. The arterial wall, however, is viscoelastic, exhibiting both the properties of an elastic solid that regains its original form after a force applied to it is removed and a viscous liquid that maintains a deformity once the deforming force is no longer applied. The arterial wall heterogeneous, with the main elastic components being collagen and elastin, connected to a variable amount of smooth muscle all held in a liquid extracellular matrix, via a recognised 3 layer macroscopic structure.

In addition it may well be that the arterial wall moves anisotropically as opposed to isotropically (i.e. differently in different directions in response to the same stress). Thus the fundamental underlying principles by which elastic theory can be applied to arterial stiffness may be flawed. However almost all research on arterial stiffness uses elastic theory as its basis.<sup>6</sup> It is considered that the viscous behaviour of the arterial wall is negligible when deforming stresses are applied slowly. If the arterial wall was examined as an anisotropic structure then a much greater amount of data would be required to be collected in order to calculate indices of arterial stiffness. As far as I am aware this is currently not practicable.

A further weakness of our study is that carotid IMT is not a measurement of entire arterial wall thickness and represents the intima and the tunica media of the carotid artery.<sup>54</sup> Both Pignoli and Wong have demonstrated that ultrasonic estimation of IMT was accurate when compared to histological verification, as long as the far wall of the insonated artery was examined, a practice that we have followed. Wong et al suggest that the adventitial layer contributes 25% of the thickness of carotid arterial walls by histological analysis, but that the adventitial layer is not recognised by ultrasound consistently or accurately.<sup>282,283</sup> Clearly the adventitial layer of an artery has important mechanical properties. In a study of human femoral arteries, Schulze-Bauer estimated that the adventitial layer carried approximately 25% of the arterial wall load.<sup>284</sup> In line with previous studies of this nature we have not attempted to correct for the lack of measurement of the adventitial layer in our estimation of



wall thickness.<sup>285</sup> A change in wall thickness of 25% or less would not alter the statistical significance of our results, and it is widely accepted that most structural change in the arterial wall in response to ageing and development of atherosclerotic disease are believed to be in the intima and media.<sup>286, 287</sup> Furthermore we used the same method of calculating the Young's modulus in all our patient groups therefore avoided introducing systematic bias.

The reproducibility of our measurements is at least as good as the best reported in the literature using conventional B-mode based techniques.<sup>240</sup> The reproducibility is improved when compared to methods using echo tracking.<sup>241</sup> The reason that the variability of our measurements of the arterial distension parameters is still around 10% is because there is a finite variability of any biological parameter, and that despite measuring the arterial distension waveform with improved resolution, we had to use brachial arterial blood pressure measurement at systole and diastole as the only information available from the APW to calculate indices of arterial stiffness. More detailed information from the APW could lead to more reproducible measurements.

When interpreting indices of arterial stiffness, in line with other studies of this nature we have used data from the left CCA only. This artery demonstrated superior reproducibility to the right in our reproducibility study.<sup>248</sup> The left CCA is anatomically the most closely related to the aorta arising directly from the aortic arch as opposed to the right CCA which arises indirectly from the aorta via the brachiocephalic

trunk. Thus the left CCA is thought to be most likely to reflect the qualities of the central aorta. It is possible that this greater reproducibility is demonstrated because this artery is more comfortable for the investigator to insonate when the subject is approached from the patient's right hand side.

Towards the end of the research period, the *Expert consensus document on arterial stiffness: methodological issues and clinical applications* was published.<sup>14</sup> We were pleased to note that the majority of the method we adopted for data collection was in accordance with this, despite carrying out the data collection before publication of this paper. This document divides methods of arterial stiffness calculation into three main types: systemic, regional and localised. The TDI based method is a localised method. This method is recognised to have the benefits of direct determination requiring no model for calculation and is best suited for mechanistic analyses in pathophysiology, pharmacology and therapeutics, rather than for epidemiological studies.<sup>14</sup> The document also recommends simultaneous measurement of local (in this case carotid) pulse pressure rather than use of brachial pressure. Previous studies have shown that using brachial artery pressure is a good substitute for carotid artery pressure when calculating carotid artery stiffness indices.<sup>288</sup> Arterial pressure does vary throughout the arterial tree due to blood pressure amplification. Translation functions are available to estimate carotid pressures from other points in the arterial tree. Recent studies suggest that these are inaccurate especially as

amplification varies with age,<sup>289</sup> and therefore brachial arterial pressure was used in this study. The expert consensus document also declares that carotid femoral PWV is the gold standard measurement of arterial stiffness based on simplicity and reproducibility, although it does also acknowledge that US based systems may provide better information regarding the arterial wall. We were pleased that the reproducibility of the TDI system is nearly as good as PWV (10.85% versus 8.3% for inter-observer reproducibility and 10.16% versus 11.0% for intra-observer reproducibility). Whilst PWV is a direct measurement, calculation requires accurate estimation of arterial length (difficult in obese subjects or those with a large bust) between two transducers, both of which have to be fitted to the subject. This is not necessarily easier or quicker to carry out than an ultrasound scan of the common carotid artery and brachial artery blood pressure measurements. In addition, with the advent of high quality compact duplex machines, portability is not an issue. It did take the principal investigator just over one year to resolve all issues in order to produce reproducible data. However the second investigator was able to acquire these skills in just a few sessions once the technique was demonstrated to him, although he did have approximately eight years experience in ultrasound scanning.

We have consistently shown that the changes in arterial stiffness are earlier or increased when compared to IMT. In some respects this is not surprising, given the likelihood that increased IMT is one of the earliest signs of atherosclerotic change. However our results do support the

premise that increased arterial stiffness is either a cause of atherosclerosis or one of the earliest indicators of its presence. This is important as there is no known treatment to reverse atherosclerosis. We need to target the development of atherosclerosis earlier than its manifestation via increased IMT or blood pressure. Understanding the relationship between arterial stiffness and atherosclerosis may be the key to this.

## **Future Studies**

Despite the fact that the TDI system is able to plot the ADW in detail, the only information we were able to use with regard to the arterial pressure waveform (APW) was systolic and diastolic pressure. We believe that the TDI method to measure the arterial distension waveform would show its full potential if coupled with effective technology that could simultaneously measure the arterial pressure waveform (APW). The most commonly used device to do this non-invasively is the Portapres ([www.finapres.com](http://www.finapres.com)). This device measures and records the APW in the digits of either hand and together with non invasive brachial blood pressure measurements is able to provide a continuous calibrated APW. An applanation tonometer could provide the same information with the advantage of potential measurement of the carotid APW. If this was to be carried out, calibration would be required, presumably using brachial blood pressure and a translation algorithm. Used in conjunction with the TDI system, a full plot of the APW and ADW would allow calculation of

the relationship between pressure and distension at all physiological pressures. The gradient of the slope would be a true pressure independent measure of arterial stiffness, and could provide information about how stiffness in the arterial wall changes across the stress of the cardiac cycle. Potentially therefore a combination of both TDI and PWV May yield very detailed information, most appropriate in a research setting. Improved reproducibility may also be demonstrated.

We were disappointed that our study looking at Ramipril and arterial stiffness in subjects with PAD failed due to recruitment and follow up issues. This was due to poor study design and time constraints, and we believe would yield important information to help explain the pressure independent cardiovascular benefits of Ramipril if carried out successfully.

## Conclusions

This thesis has demonstrated that the TDI-based system produces a high resolution plot of the ADW that has good reproducibility when used in conjunction with brachial blood pressure to calculate indices of arterial stiffness. We have shown that indices of structural and material arterial wall stiffness are more increased than IMT in studies comparing healthy subjects to those with PAD and CKD and with ageing. This suggests that increased arterial stiffness may be more than just a marker of cardiovascular disease that actually changes before atherosclerosis develops.

# APPENDIX 1 :DERIVATION OF YOUNG'S MODULUS

## Pete Hoskin's opinion

Dear Martin

As we have agreed,

$$E_p (\text{pressure strain}) = (P_s - P_d) / [(D_s - D_d)/D_d] \quad (1)$$

units are those of pressure is Pa (N/m<sup>2</sup>)

McDonalds 1974, table 10.6

gives an equation as part of its header, which is that

$$E (\text{Youngs}) = E_p (\text{pressure strain}) * (R/h) \quad (2)$$

Units are the same as  $E_p$ ; ie Pa (N/m<sup>2</sup>)

McDonald 5th edition

$$E = E_p * (1/h)$$

$$\text{Units are Pa/m} \quad (3)$$

[http://en.wikipedia.org/wiki/Young\\_Modulus](http://en.wikipedia.org/wiki/Young_Modulus)

Youngs modulus is  $E = \text{stress} / \text{strain}$

$$\text{Or } E = (F/A)/(x/l) \quad (4)$$

units are Pa

### Discussion

The units are a quick way to decide which is possibly correct and which is clearly wrong. Equation 4 shows the correct units, so equation 2 might be right, equation 3 is definitely wrong.

In referencing this in your paper, I suggest that we forget about a history of definition and simply quote the article by Reneman. I would also not get into a discussion on previous definitions which have been wrong. This might come up at another time, but is not connected with our article, and we do not want to deflect or confuse the referees. Reneman is one of the major players in this general area; in a search I recently found some 350 papers to his name!

The maths that I provided last time was based on the article by Reneman, and the only difference between this and McDonald 1974 is a factor of 2. I have a grant to get in on Monday but I have forwarded this to Kate Fraser who you met who I will ask to check over the basic maths and we will get back to you on Tuesday or Wednesday this week.

If you note the table in McDonald 5<sup>th</sup> edition looks to be a summary of some committee consensus. I am bit surprised that they made a mistake, but having done chapters in books, trying to tie down every



single equation is painful and there will be mistakes. Obviously we have found one.

Best wishes

Pete Hoskins

## Kate Frasers Opinion

Hi Martin,

Pete is correct in that

$$E = E_p * r / h$$

I think the definition of  $E$  in McDonald 5th ed is based on a different definition of stress (using a thin walled approximation of the artery) and so probably isn't strictly wrong but is best avoided.

I've come up with a straightforward derivation of  $E$  which shows how it relates to  $E_p$  - attached.

(Pete - it might be a good idea if you check this through in case I've made any mistakes.)

Hope that helps,

Kate

## Kate Fraser's Derivation

### Relationship Between Young's Modulus and Pressure Modulus

Treat the artery as a simple cylinder as shown in figure 1.

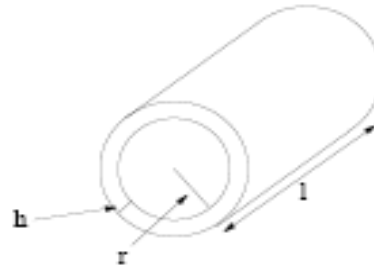


Figure 1: A cylinder inflated to pressure  $P$ , with radius  $r$ , thickness  $h$ , and length  $l$

The tangential (or hoop) stress,  $s$ , can be calculated by slicing the cylinder longitudinally and equating forces as shown in figure 2.

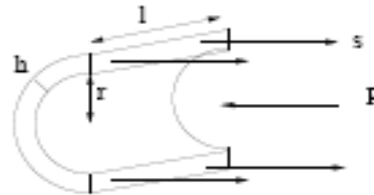


Figure 2: To calculate the stress in the wall set the force due to stress equal to the pressure force

The force in the wall is given by the stress,  $s$  multiplied by the longitudinal cross sectional area of the cylinder  $A = 2hl$ . The force due to the stress is equal to the pressure acting over the area of the longitudinal cross section and in the opposite direction.

$$\begin{aligned} 2shl &= 2Pr l \\ s &= \frac{Pr}{h} \end{aligned} \quad (1)$$

The difference in the stress at systolic pressure and the stress at diastolic pressure is then given by:

$$\Delta s = \frac{P_s r_s}{h} - \frac{P_d r_d}{h} \quad (2)$$

Strictly  $h$  will be smaller at systolic pressure than at diastolic pressure but this difference is small and can be ignored.

The strain,  $\epsilon$ , is given by the difference in the circumference at systolic and diastolic pressure and divided by the diastolic circumference to get a dimensionless number.

$$\begin{aligned}\epsilon &= \frac{C_s - C_d}{C_d} \\ C &= 2\pi r \\ \epsilon &= \frac{2\pi(r_s - r_d)}{2\pi r_d} \\ &= \frac{r_s - r_d}{r_d}\end{aligned}\tag{3}$$

Young's modulus,  $E_y$ , is then the ratio of stress to strain which is

$$\begin{aligned}E_s &= \frac{s}{\epsilon} \\ E_s &= \frac{r_d(P_s r_s - P_d r_d)}{h(r_s - r_d)}\end{aligned}\tag{4}$$

But the systolic radius is just the diastolic radius plus the change in radius  $r_s = r_d + \Delta r$ . Or, if the change in radius is small compared to the actual radius,  $\Delta r \ll r_d$ , then  $r_s \approx r_d$  and:

$$\begin{aligned}E_y &\approx \frac{r_d(P_s r_d - P_d r_d)}{h(r_s - r_d)} \\ &= \frac{r_d^2(P_s - P_d)}{h(r_s - r_d)}\end{aligned}\tag{5}$$

The pressure strain elastic modulus is *defined* as:

$$E_p = \frac{r_d(P_s - P_d)}{r_s - r_d}\tag{6}$$

And finally, writing  $E_y$  in terms of  $E_p$  gives:

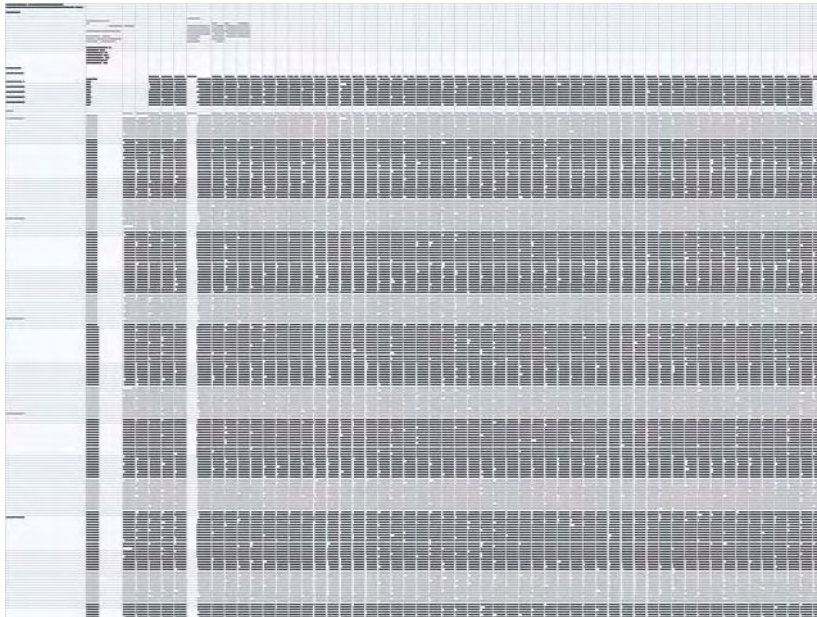
$$E_y = \frac{E_p r_d}{h}\tag{7}$$

## APPENDIX 2 : OFFLINE COMPUTER ANALYSIS OF ARTERIAL SCANS

### Spreadsheet

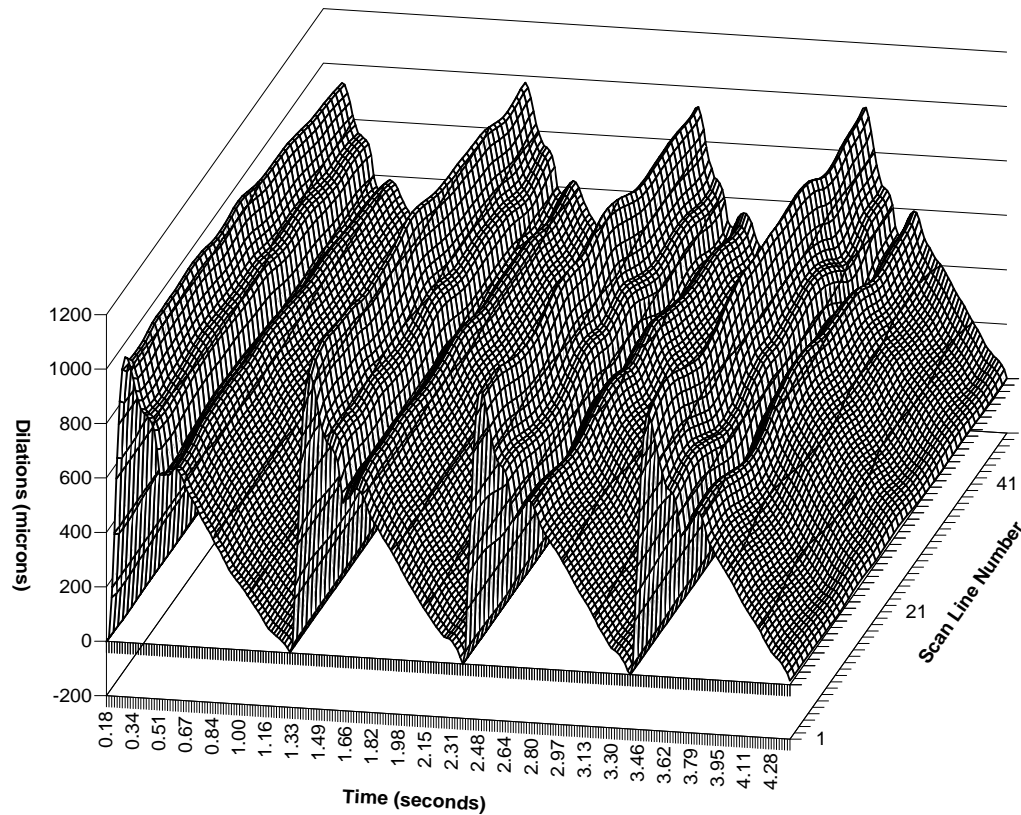
Each scan acquisition provided a data plot of over 20000 points as demonstrated in Figure 35.

**Figure 35:** excel spreadsheet containing over 20000 data points from one scan of the CCA



These data are shown graphically in Figure 36.

**Figure 36:** A graphical representation of arterial distension waveform



**Legend:** Dilations = how far in microns arterial wall moved apart during cardiac cycle; Scan line number= virtual line down which software calculated arterial wall dilatation (48 line in total); Time = total length in time of scan and scale over which arterial dilatation occurred. This data was captured over 4 cardiac cycles from a segment of common carotid artery measuring approximately 2cm.

Clearly this data needed summarising in order to be useful. This was done using a macro within Microsoft excel written using visual basic and is reproduced in the next section. The function of this macro is to summarise the data shown in Figures 35 and 36 and present it as ADC per cardiac cycle and per scan, together with between cycle variability within each scan.

## ***Analysis Macro***

```
Sub ANALYSIS()  
'  
' ANALYSIS Macro  
' Macro recorded 10/06/2004 by Martin Claridge  
" Keyboard Shortcut: Ctrl+q  
' Range("H6").Select  
ActiveCell.FormulaR1C1 = "ANALYSIS"  
Range("H9").Select  
ActiveCell.FormulaR1C1 = "CARDIAC CYCLE 1"  
Range("H10").Select  
ActiveCell.FormulaR1C1 = "CARDIAC CYCLE 2"  
Range("H11").Select  
ActiveCell.FormulaR1C1 = "CARDIAC CYCLE 3"  
Range("H12").Select  
ActiveCell.FormulaR1C1 = "CARDIAC CYCLE 4"  
Columns("I:I").Select  
Range("I4").Activate  
Columns("H:H").EntireColumn.AutoFit  
Range("I8").Select  
ActiveCell.FormulaR1C1 = "MAX"  
Range("J8").Select  
ActiveCell.FormulaR1C1 = "MIN"  
Range("H13").Select  
ActiveCell.FormulaR1C1 = "AVERAGE"  
Range("H14").Select  
ActiveCell.FormulaR1C1 = "STD DEV"  
Range("H15").Select  
ActiveCell.FormulaR1C1 = "VARINCE"  
Range("H15").Select  
ActiveCell.FormulaR1C1 = "VARIANCE"  
Range("I9").Select  
ActiveCell.FormulaR1C1 = "=AVERAGE(R[21]C:R[21]C[37])"  
Range("I10").Select  
ActiveWindow.SmallScroll ToRight:=-5  
Range("I10").Select  
ActiveCell.FormulaR1C1 = "=AVERAGE(R[22]C:R[22]C[37])"  
Range("I11").Select  
ActiveWindow.SmallScroll ToRight:=-2  
Range("I11").Select  
ActiveCell.FormulaR1C1 = "=AVAERAGE(R[23]C:R[23]C[37])"  
Range("I11").Select  
ActiveCell.FormulaR1C1 = "=AVERAGE(R[23]C:R[23]C[37])"  
Range("I12").Select  
ActiveCell.FormulaR1C1 = "=AVERAGE(R[24]C:R[24]C[37])"  
Range("I13").Select  
ActiveCell.FormulaR1C1 = "=AVERAGE(R[-4]C:R[-1]C)"  
Range("I13").Select  
ActiveWindow.SmallScroll Down:=5  
ActiveWindow.SmallScroll ToRight:=-6  
ActiveWindow.SmallScroll Down:=-3  
Range("J9").Select  
ActiveCell.FormulaR1C1 = "=AVERAGE(R[22]C[-1]:R[22]C[36])"  
Range("J10").Select  
ActiveCell.FormulaR1C1 = "=avarage(R[23]C[-1]:R[23]C[36])"
```

```

Range("J10").Select
ActiveCell.FormulaR1C1 = "=AVERAGE(R[23]C[-1]:R[23]C[36])"
Range("J11").Select
ActiveCell.FormulaR1C1 = "=AVERAGE(R[24]C[-1]:R[24]C[36])"
Range("J12").Select
ActiveCell.FormulaR1C1 = "=AVERAGE"
Range("J12").Select
ActiveCell.FormulaR1C1 = "=AVERAGE(R[25]C[-1]:R[25]C[36])"
Range("J13").Select
ActiveCell.FormulaR1C1 = "=AVERAGE(R[-4]C:R[-1]C)"
Range("K8").Select
ActiveCell.FormulaR1C1 = "difference"
Range("K9").Select
ActiveCell.FormulaR1C1 = "=RC[-2]-RC[-1]"
Range("K9").Select
Selection.AutoFill Destination:=Range("K9:K13"), Type:=xlFillDefault
Range("K9:K13").Select
Range("I14").Select
ActiveCell.FormulaR1C1 = "=STDEV(R[-5]C[2]:R[-2]C[2])"
Range("I15").Select
ActiveCell.FormulaR1C1 = "=R[-1]C/R[-2]C"
Range("I15").Select
Selection.NumberFormat = "0.00%"
ActiveWindow.SmallScroll Down:=-4
End Sub

```

### ***Summary produced by analysis macro***

Figure 37 details the result produced by the analysis macro which was then used in further calculations.

**Figure 37:** Results of analysis Macro.

ANALYSIS			
	MAX	MIN	difference
CARDIAC CYCLE 1	319.2942	-1.97011	321.2643
CARDIAC CYCLE 2	324.1096	-0.36886	324.4784
CARDIAC CYCLE 3	315.8209	0.396667	315.4243
CARDIAC CYCLE 4	310.639	0.11858	310.5204
AVERAGE	317.4659	-0.45593	317.9218
STD DEV	6.196246		
VARIANCE	1.95%		



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